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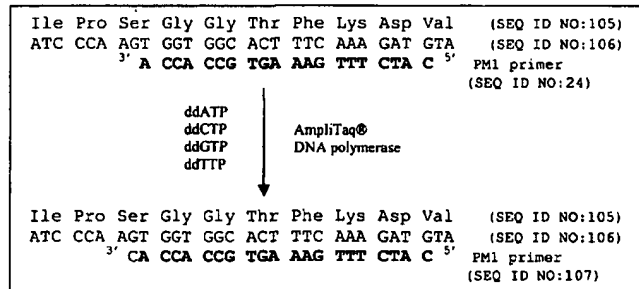
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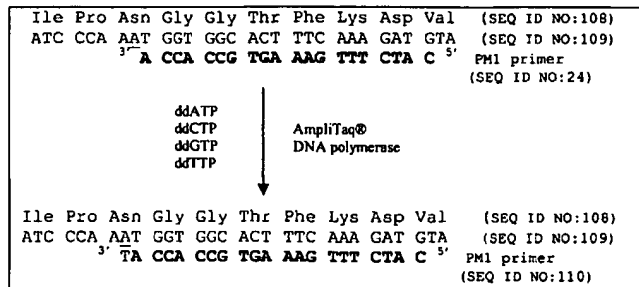
"PM1" Test

AHAS1

'Topas'



'PM1'



(57) Abstract: The present invention provides compositions and methods for assaying commercially relevant imidazolinone herbicide tolerance conferred by a *Brassica napus* AHAS1 PM1 mutation and a *Brassica napus* AHAS3 PM2 mutation in a plant.



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COMPOSITIONS AND METHODS FOR IDENTIFYING PLANTS HAVING INCREASED TOLERANCE TO IMIDAZOLINONE HERBICIDES

BACKGROUND OF THE INVENTION

Field of the Invention

[001] This invention relates generally to compositions and methods for identifying *Brassica* plants having increased tolerance to an imidazolinone herbicide.

Background Art

[002] Canola is the seed derived from any of the *Brassica* species *B. napus*, *B. campestris/rapa*, and certain varieties of *B. juncea*. Canola oil is high in monounsaturated fats, moderate in polyunsaturated fats, and low in saturated fats, having the lowest level of saturated fat of any vegetable oil. Thus canola oil is an important dietary option for lowering serum cholesterol in humans. In addition, the protein meal which is the byproduct of canola oil production has a high nutritional content and is used in animal feeds.

[003] Imidazolinone and sulfonylurea herbicides are widely used in modern agriculture due to their effectiveness at very low application rates and relative non-toxicity in animals. Both of these herbicides act by inhibiting acetohydroxyacid synthase (AHAS; EC 4.1.3.18, also known as acetolactate synthase or ALS), the first enzyme in the synthetic pathway of the branched chain amino acids valine, leucine and isoleucine. Several examples of commercially available imidazolinone herbicides are PURSUIT® (imazethapyr), SCEPTER® (imazaquin) and ARSENAL® (imazapyr). Examples of sulfonylurea herbicides are chlorsulfuron, metsulfuron methyl, sulfometuron methyl, chlorimuron ethyl, thifensulfuron methyl, tribenuron methyl, bensulfuron methyl, nicosulfuron, ethametsulfuron methyl, rimsulfuron, triflurosulfuron methyl, triasulfuron, primisulfuron methyl, cinosulfuron, amidosulfuron, fluzasulfuron, imazosulfuron, pyrazosulfuron ethyl and halosulfuron.

[004] Due to their high effectiveness and low toxicity, imidazolinone herbicides are favored for application to many crops, including canola, by spraying over the top of a wide area of vegetation. The ability to spray an herbicide over the top of a wide range of vegetation decreases the costs associated with plantation establishment and maintenance and

decreases the need for site preparation prior to use of such chemicals. Spraying over the top of a desired tolerant species also results in the ability to achieve maximum yield potential of the desired species due to the absence of competitive species. However, the ability to use such spray-over techniques is dependent upon the presence of imidazolinone resistant species of the desired vegetation in the spray over area. In addition, because residual imidazolinones persist in a sprayed field, a variety of resistant species is advantageous for crop rotation purposes.

[005] Unfortunately, the *Brassica* species which are the source of canola are closely related to a number of broad leaf cruciferous weeds, for example, stinkweed, ball mustard, wormseed mustard, hare's ear mustard, shepherd's purse, common peppergrass, flixweed, and the like. Thus it was necessary to develop *Brassica* cultivars which are tolerant or resistant to the imidazolinone herbicides. Swanson, *et al.* (1989) *Theor. Appl. Genet.* 78, 525-530 discloses *B. napus* mutants P₁ and P₂, developed by mutagenesis of microspores of *B. napus* (cv 'Topas'), which demonstrated tolerance to the imidazolinone herbicides PURSUIT® and ASSERT® at levels approaching ten times the field-recommended rates. The homozygous P₂ mutant produced an AHAS enzyme which was 500 times more tolerant to PURSUIT® than wild type enzyme, while the AHAS enzyme from the homozygous P₁ mutant was only slightly more tolerant than the wild type enzyme. In field trials, the P₁, P₂, and P₁ x P₂ hybrid withstood ASSERT® applications up to 800 g/ha with no loss of yield. The P₁ and P₂ mutations were unlinked and semidominant, and P₁ x P₂ crosses tolerated levels of PURSUIT® higher than those tolerated by either homozygous mutant. Imidazolinone-tolerant cultivars of *B. napus* were developed from the P₁ x P₂ mutants and have been sold as CLEARFIELD® canola. See also, Canadian patent application number 2,340,282; Canadian patent number 1,335,412, and European patent number 284419.

[006] Rutledge, *et al.* (1991) *Mol. Gen. Genet.* 229, 31-40) discloses the nucleic acid sequence of three of the five genes encoding AHAS isoenzymes in *B. napus*, *AHAS1*, *AHAS2*, and *AHAS3*. Rutledge, *et al.* discusses the mutants of Swanson, *et al.* and predicts that the two alleles that conferred resistance to imidazolinone herbicides correspond to *AHAS1* and *AHAS3*. Hattori *et al.* (1995) *Mol. Gen. Genet.* 246, 419-425 disclose a mutant allele of *AHAS3* from a mutant *B. napus* cv Topas cell suspension culture line in which a single nucleotide change at codon 557 leading to an amino acid change from tryptophan to leucine confers resistance to sulfonylurea, imidazolinone, and triazolopyrimidine herbicides. Codon 557 of Hattori, *et al.* corresponds to codon 556 of the *AHAS3* sequence disclosed in

Rutledge, *et al.*, *supra*, and to codon 556 of the *AHAS3* sequence set forth as GENBANK accession number gi/17775/emb/Z11526/.

[007] A single nucleotide mutation at codon 173 in a *B. napus* ALS gene, corresponding to *AHAS2* of Rutledge *et al.*, *supra*, leads to a change from Pro to Ser (Wiersma *et al.* (1989) *Mol. Gen. Genet.* 219, 413-420). The mutant *B. napus* *AHAS2* gene was transformed into tobacco to produce a chlorsulfuron tolerant phenotype.

[008] U.S.Pat.Nos. 6,114,116 and 6,358,686 disclose nucleic acid sequences from *B. napus* and *B. oleracea* containing polymorphisms, none of which appears to correspond to the polymorphism disclosed in Hattori, *et al.*, *supra*.

[009] For commercially relevant *Brassica* cultivars, it is necessary to ensure that each lot of herbicide-resistant seed contains all mutations necessary to confer herbicide tolerance. A method is needed to detect mutations in *Brassica* *AHAS1* and *AHAS3* genes that confer increased imidazolinone tolerance to commercial cultivars.

SUMMARY OF THE INVENTION

[010] The present invention describes the location and identity of a single nucleotide polymorphism at position 1937 of the *AHAS1* gene of *B. napus*, the polymorphism being designated as the PM1 mutation. The PM1 mutation confers about 15% of the tolerance to imidazolinone herbicides that is present in CLEARFIELD® canola. CLEARFIELD® canola also contains a second single nucleotide polymorphism at position 1709 of the *AHAS3* gene of *B. napus*, which corresponds to the tryptophan to leucine substitution described in Hattori *et al.*, *supra*. For the purpose of the present invention, this polymorphism is designated as the PM2 mutation. The PM2 mutation confers about 85% of the tolerance to imidazolinone herbicides exhibited by CLEARFIELD® canola. Both the PM1 and PM2 mutations are required to produce a *Brassica* plant with sufficient herbicide tolerance to be commercially relevant, as in CLEARFIELD® canola.

[011] Accordingly, the present invention provides methods of identifying a plant having increased tolerance to an imidazolinone herbicide by detecting the presence or absence of the *B. napus* PM1 and PM2 mutations in the plant. One of the advantages of the present invention is that it provides a reliable and quick means to detect plants with commercially relevant imidazolinone tolerance.

[012] In one embodiment, the invention provides a method of assaying a plant for imidazolinone herbicide resistance conferred by the combination of the PM1 mutation of the *B. napus* *AHAS1* gene and the PM2 mutation of the *B. napus* *AHAS3* gene. In this method,

genomic DNA is isolated from the plant, the presence or absence of the PM1 mutation is determined, and the presence or absence of the PM2 mutation is determined, wherein the presence of the PM1 mutation and the PM2 mutation is indicative of commercially relevant imidazolinone tolerance in the plant.

[013] In another embodiment, the invention provides novel polynucleotide primers useful for detecting the PM1 and PM2 mutations.

BRIEF DESCRIPTION OF THE DRAWINGS

[014] Figure 1A shows the nucleic acid and amino acid sequences of *B. napus AHAS1* containing the PM1 mutation (SEQ ID NO:1 and SEQ ID NO:101, respectively).

[015] Figure 1B shows the nucleic acid and amino acid sequences of *B. napus AHAS3* containing the PM2 mutation (SEQ ID NO:2 and SEQ ID NO:102, respectively).

[016] Figure 1C shows the nucleic acid and amino acid sequences of wild type *B. napus* cv. 'Topas' *AHAS1* (SEQ ID NO:3 and SEQ ID NO:103, respectively).

[017] Figure 1D shows the nucleic acid and amino acid sequences of wild type *B. napus AHAS3* Topas cv. (SEQ ID NO:4 and SEQ ID NO:104, respectively).

[018] Figure 1E is a table setting forth the sequences of various oligonucleotides (SEQ ID NOs: 5-88) useful in determining the presence or absence of the PM1 and PM2 mutations in accordance with the invention.

[019] Figure 2 is a schematic representation of one embodiment of the PM1 mutation determination step of a primer extension-based assay of the invention. The coding strand is shown with the amino acid translation of the codons. The wild type plant is denoted as 'Topas' (SEQ ID NOs: 105, 106, 24, 105, 106, and 107, respectively, in order of appearance) and the mutated plant is denoted as 'PM1' (SEQ ID NOs: 108, 109, 24, 108, 109, and 110, respectively, in order of appearance). The mutated nucleotide "A" is underlined on the coding strand. The PM1 extension primer is indicated in bold and is placed at its annealing site on *AHAS1*.

[020] Figure 3 is a schematic representation of one embodiment of the PM2 mutation determination step of a primer extension-based assay of the invention. The coding strand is shown with the amino acid translation of the codons. The wild type plant is denoted as 'Topas' (Seq ID NOs: 111, 112, 66, 111, 112, and 113, respectively, in order of appearance) and the mutated plant is denoted as 'PM2' (SEQ ID NOs: 114, 115, 66, 114, 115, and 116, respectively, in order of appearance). The mutated nucleotide "T" is

underlined on the coding strand. The PM2 extension primer is indicated in bold and is placed at its annealing site on *AHAS3*.

[021] Figure 4 is a table describing the predicted phenotypes of double haploid *B. napus* plants used to validate the method of the invention.

[022] Figure 5 is a table describing the results of the method of the invention in an embodiment employing the ABI PRISM® SNP detection system.

[023] Figure 6 is a table describing the results of the method of the invention in an embodiment employing the PYROSEQUENCING™ detection system.

DETAILED DESCRIPTION OF THE INVENTION

[024] The present invention provides methods and compositions for identifying plants having increased tolerance to an imidazolinone herbicide by virtue of the presence of the *B. napus* PM1 and PM2 mutations. More particularly, the methods and compositions of the present invention allow identification of *Brassica* seeds and plants having commercially relevant imidazolinone tolerance, such as CLEARFIELD® canola. In some embodiments, the methods of the invention employ novel polynucleotide primers including PM1 extension primers and PM2 extension primers.

[025] It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized.

[026] For the purposes of the present invention, the level of tolerance to imidazolinone herbicides exhibited by CLEARFIELD® canola which contains both the PM1 and PM2 mutations is defined as 100% tolerance, or "commercially relevant imidazolinone tolerance" or "commercial field tolerance". The terms "tolerance" and "resistance" are used interchangeably herein.

[027] "Homologs" are defined herein as two nucleic acids or polypeptides that have similar, or "identical", nucleotide or amino acid sequences, respectively. Homologs include allelic variants, analogs, orthologs and paralogs. As used herein, the term "allelic variant" refers to a nucleotide sequence containing polymorphisms that lead to changes in the amino acid sequences of AHAS proteins and that exist within a natural population (e.g., a plant species or variety). As used herein, the term "analogs" refers to two nucleic acids that have the same or similar function, but that have evolved separately in unrelated organisms. The term "orthologs" refers to two nucleic acids from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides

having the same or similar functions. As also used herein, the term "paralogs" refers to two nucleic acids that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related (Tatusov, R.L. *et al.*, 1997. Science 278(5338):631-637).

[028] As defined herein, a "PM1 mutation" refers to a single nucleotide polymorphism in a *B. napus AHAS1* gene in which there is a "G" to "A" nucleotide substitution at position 1937 of the *AHAS1* wildtype polynucleotide sequence shown in Figure 1C (SEQ ID NO:3) or at a nucleotide position that corresponds to position 1937 in an *AHAS1* homolog, which substitution leads to a serine to asparagine amino acid substitution at position 638 in the *B. napus AHAS1* enzyme.

[029] A "PM1 oligonucleotide" refers to an oligonucleotide sequence corresponding to a PM1 mutation. An oligonucleotide as defined herein is a nucleic acid comprising from about 8 to about 25 covalently linked nucleotides. In accordance with the invention, an oligonucleotide may comprise any nucleic acid, including, without limitation, phosphorothioates, phosphoramidates, peptide nucleic acids, and the like. As defined herein, "corresponding to a PM1 mutation" includes the following: an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 5' of position 1937 of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising any one of SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; or SEQ ID NO:23 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 3' of position 1937 of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising any one of SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; or SEQ ID NO:42 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of the *AHAS1* gene which spans position 1937 of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising SEQ ID NO:45 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 5' of position 1937 of the complement of the *AHAS1* gene set forth in SEQ ID NO:3; an oligonucleotide capable of specific hybridization to a region of an *AHAS1* gene which is 3' of position 1937 of the

complement of the *AHAS1* gene set forth in SEQ ID NO:3; and an oligonucleotide capable of specific hybridization to a region of the *AHAS1* gene which spans position 1937 of the complement of the *AHAS1* gene as set forth in SEQ ID NO:3 (for example, an oligonucleotide comprising SEQ ID NO: 46 as set forth in Figure 1E). The term "nucleic acid" includes RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. These terms also encompass RNA/DNA hybrids.

[030] As defined herein, a "PM2 mutation" refers to a single nucleotide polymorphism in a *B. napus AHAS3* gene in which there is a "G" to "T" nucleotide substitution at position 1709 of the *AHAS3* wildtype polynucleotide sequence shown in Figure 1D (SEQ ID NO:4) or at a nucleotide position that corresponds to position 1709 in an *AHAS3* homolog, which substitution leads to a tryptophan to leucine amino acid substitution at position 556 in the *B. napus AHAS3* enzyme.

[031] A "PM2 oligonucleotide" refers to an oligonucleotide sequence corresponding to a PM2 mutation. As defined herein, "corresponding to a PM2 mutation" includes the following: an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 5' of position 1709 of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising any one of SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58; SEQ ID NO:59; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; or SEQ ID NO:65 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 3' of position 1709 of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising any one of SEQ ID NO:66; SEQ ID NO:67; SEQ ID NO:68; SEQ ID NO:69; SEQ ID NO:70; SEQ ID NO:71; SEQ ID NO:72; SEQ ID NO:73; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:77; SEQ ID NO:78; SEQ ID NO:79; SEQ ID NO:80; SEQ ID NO:81; SEQ ID NO:82; SEQ ID NO:83; and SEQ ID NO:84 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of the *AHAS3* gene which spans position 1709 of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising SEQ ID NO: 85 as set forth in Figure 1E); an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 5' of position 1709 of the complement of the *AHAS3* gene set forth in SEQ ID NO:4; an oligonucleotide capable of specific hybridization to a region of an *AHAS3* gene which is 3' of position 1709 of the complement of the *AHAS3* gene set forth in SEQ ID NO:4; and an oligonucleotide capable of specific hybridization to a region of the *AHAS3*

gene which spans position 1709 of the complement of the *AHAS3* gene as set forth in SEQ ID NO:4 (for example, an oligonucleotide comprising SEQ ID NO: 86 as set forth in Figure 1E).

[032] Also encompassed in the present invention are oligonucleotides corresponding to the wild type alleles at the PM1 and PM2 mutations which are useful as controls in the SNP detection assays. For example, an oligonucleotide corresponding to position 1937 of the *AHAS1* gene set forth in SEQ ID NO:1, comprising a sequence selected from the group consisting of SEQ ID NO:43 and SEQ ID NO:44 as set forth in Figure 1E, is useful as a control in a SNP assay for the PM1 mutation. Similarly, an oligonucleotide corresponding to position 1709 of the *AHAS3* gene set forth in SEQ ID NO:2, comprising a sequence selected from the group consisting of SEQ ID NO:85 and SEQ ID NO:86 as set forth in Figure 1E, is useful as a control in a SNP assay for the PM2 mutation.

[033] The presence of the PM1 and PM2 mutations in a plant may confer tolerance to such imidazolinone herbicides as PURSUIT[®] (imazethapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), CADRE[®] (imazapic, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid), RAPTOR[®] (imazamox, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid), SCEPTER[®] (imazaquin, 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-quinolinecarboxylic acid), ASSERT[®] (imazethabenz, methyl esters of 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-4-methylbenzoic acid and 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methylbenzoic acid), ARSENAL[®] (imazapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid), and the like. In addition, the PM1 and PM2 mutations may confer resistance to sulfonylurea and triazolopyrimidine herbicides.

[034] The PM1 and PM2 mutations may be present in a plant by virtue of mutagenesis of any species of plant containing the *B. napus AHAS1* and *AHAS3* genes, respectively. Alternatively, the PM1 and PM2 mutations may be present in a plant by virtue of transformation of the *B. napus AHAS1* PM1 gene and the *B. napus AHAS3* PM2 genes into the plant, using known methods such as those set forth in U.S.Pat.Nos. 5,591,616; 5,767,368; 5,736,369; 6,020,539; 6,153,813; 5,036,006; 5,120,657; 5,969,213; 6,288,312; 6,258,999, and the like. Preferably, the plant is a *Brassica* oilseed. More preferably, the plant species is selected from the group consisting of *B. napus*, *B. campestris/rapa*, and *B. juncea*. Most

preferably, the plant species is *B. napus*. In accordance with the present invention, the term "plant" includes seeds, leaves, stems, whole plants, organelles, cells, and tissues.

[035] In the first step of the method of the invention, genomic DNA is isolated from the plant. It is to be understood that when practicing the method of the present invention, genomic DNA can be extracted from the plant by any method known to those of skill in the art. Genomic DNA can be extracted from a whole plant, a plant leaf, a plant stem, a plant seed, or any plant organelle, cell or tissue. One non-limiting method for extracting the DNA from a plant leaf is described in Example 1 below.

[036] In the second step of the method of the invention, the presence or absence of the PM1 mutation in the extracted DNA is determined. In the third step of the invention, the presence or absence of the PM2 mutation in the extracted DNA is determined. In accordance with the invention, the steps of detecting the PM1 and PM2 mutations may be performed in any order, or simultaneously.

[037] Any method may be used to detect the PM1 and PM2 mutations. For example, commercially available single nucleotide polymorphism (SNP) detection systems may be used, such as the SNP-IT™ system (Orchid Biosciences, Princeton, NJ), the MassArray™ System (Sequenom, Inc., San Diego, CA), the BeadArray™ System (Illumina, San Diego, CA), the ABIPrism Genetic Analyzer (Applied Biosystems, Foster City, CA), the ALFexpress™ (Amersham Biosciences, Buckinghamshire, UK), the PSQ™96 System (Pyrosequencing AB, Uppsala, Sweden), the Invader™ assay (Third Wave Agbio, Inc., Madison, WI), and the like. A variety of methods exist for identification of a nucleotide at a polymorphic site in a nucleic acid, as described, for example, in U.S.Pat.Nos. 6,087,095; 6,046,005; 6,017,702; 5,981,186; 5,976,802; 5,928,906; 5,912,118; 5,908,755; 5,869,242; 5,853,979; 5,849,542; 5,834,189; 4,851,331; 4,656,127; 5,679,524; 6,004,744; 6,013,431; 6,210,891; 6,183,958; 5,958,692; 5,851,770; 6,110,684; 5,856,092; 5,605,798; 5,547,835; 6,194,144; 6,043,031; 6,322,980; 6,340,566, and the like. Such technologies include, but are not limited to, allele-specific primer extension, allele-specific hybridization, allele-specific ligation, allele-specific enzymatic cleavage, mismatch detection using resolvase, and sequencing. These technologies can be combined with different signal detection technologies such as fluorescence, fluorescence resonance energy transfer, fluorescence polarization, luminescence and mass spectroscopy.

[038] In some embodiments of the method of the invention, the isolated DNA is combined with a PM1 extension primer and a PM2 extension primer, as defined below, in the presence of one or more SNP detection reagents, thereby creating a detection product. The

detection product is then examined to determine the presence or absence of a PM1 mutation or a PM2 mutation in the isolated DNA. As used herein, the term "SNP detection reagent" refers to any reagent that is part of any SNP technology, technique or kit that can be used to detect single nucleotide polymorphisms.

[039] In one embodiment, the template DNA is combined with a first extension primer which is suitable for detection of a PM1 mutation, a second extension primer suitable for detection of a PM2 mutation, and one or more SNP detection reagents. An "extension primer" is an oligonucleotide that binds to the target DNA upstream from the target mutation in the direction of extension. In accordance with the invention, a PM1 extension primer comprises an oligonucleotide corresponding to a PM1 mutation. Similarly, a PM2 extension primer comprises an oligonucleotide corresponding to a PM2 mutation. The extension primer will preferably have a length from about 12 nucleotides to about 100 nucleotides, and more preferably have a length from about 18 nucleotides to about 60 nucleotides.

[040] The extension primer may be chosen to bind substantially uniquely to a target sequence containing a PM1 or PM2 mutation under the conditions of primer extension, so that the sequence will normally be one that is conserved or the primer is long enough to bind in the presence of a few mismatches, usually fewer than about 10% mismatches. By knowing the sequence that is upstream from the PM1 or PM2 mutation, one can select a sequence that has a high G-C ratio, so as to have a high binding affinity for the target sequence. In addition, the extension primer should bind reasonably close to the PM1 or PM2 mutation, preferably not more than about 200 nucleotides away, more preferably not more than about 100 nucleotide away, and most preferably within 50 nucleotides. In a preferred embodiment, the extension primer binds between 1 and 5 nucleotides away from the PM1 or PM2 mutation.

[041] Both the PM1 extension primer and the PM2 extension primer described herein are preferred extension primers. In one embodiment of the present invention, the PM1 extension primer comprises a sequence as shown in SEQ ID NO:24, or any contiguous primer, noncontiguous primer or homologous primer thereof. In another or further embodiment of the present invention, the PM2 extension primer comprises a sequence as shown in SEQ ID NO:66, or any contiguous primer, noncontiguous primer or homologous primer thereof. The PM1 or PM2 primer can also comprise an RNA version of any of the aforementioned extension primers.

[042] The term "contiguous primer" refers to a polynucleotide sequence that contains at least a fragment of the polynucleotide sequence of SEQ ID NO:24, SEQ ID

NO:66, SEQ ID NO:23 or SEQ ID NO:65. In one embodiment, the contiguous primer contains a 5' or 3' fragment of SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65 in addition to one or more nucleotides complementary to upstream or downstream PM1 or PM2 polynucleotide sequences. For example, a contiguous primer of the PM1 primer shown in SEQ ID NO:24 could comprise a nucleotide sequence of TAC ATCTTTGAAAGTGCCA (SEQ ID NO:89). The term "noncontiguous primer" refers to a sequence that is not contiguous with a PM1 or PM2 primer (i.e., a contiguous fragment of the PM1 or PM2 primer), but which sequence contains portions of a PM1 or PM2 primer sequence sufficient to provide the amplification or detection results obtained with SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65. For example, with reference to Figure 1E, oligonucleotides having SEQ ID NOs: 5-21 are noncontiguous with the PM1 primer having SEQ ID NO:23. Finally, the term "homologous primer" refers to a polynucleotide sequence that is substantially homologous with SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65 or a contiguous primer thereof. In a preferred embodiment, the contiguous, non-contiguous or homologous primer has the attributes of an extension primer as described above, and more preferably, binds immediately upstream or downstream from a PM1 or PM2 mutation.

[043] Substantially homologous primers included in the present invention are those that provide detection results in ranges similar to those obtained with the oligonucleotide sequence shown in SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65. In a preferred embodiment, a primer substantially homologous to SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65 is at least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-75%, 75-80%, 80-85%, 85-90% or 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more identical to an entire oligonucleotide sequence shown in SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65.

[044] To determine the percent sequence identity of two polynucleotide sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one polynucleotide for optimal alignment with the other polynucleotide). The polynucleotides at corresponding positions are then compared. When a position in one sequence (e.g., a sequence of SEQ ID NO:24, SEQ ID NO:66, SEQ ID NO:23 or SEQ ID NO:65) is occupied by the same nucleotide as the corresponding position in the other sequence, then the molecules are identical at that position. Accordingly, the percent sequence identity between the two sequences is a function of the number of identical

positions shared by the sequences (i.e., percent sequence identity = numbers of identical positions/total numbers of positions x 100). For the purposes of the invention, the percent sequence identity between two nucleic acid or polypeptide sequences is determined using the Vector NTI 6.0 (PC) software package (InforMax, 7600 Wisconsin Ave., Bethesda, MD 20814). A gap opening penalty of 15 and a gap extension penalty of 6.66 are used for determining the percent identity of two nucleic acids. A gap opening penalty of 10 and a gap extension penalty of 0.1 are used for determining the percent identity of two polypeptides. All other parameters are set at the default settings. It is to be understood that for the purposes of determining sequence identity when comparing a DNA sequence to an RNA sequence, a thymidine nucleotide is equivalent to a uracil nucleotide.

[045] The methods described in the examples employ the coding sequences of the PM1 and PM2 mutations as templates, but the method works equally well with SNP detection assays using the non-coding sequence and the primers. For example, a PM1 extension primer with the non-coding strand as template (5'TGTGTTACCGATGATCCCAA³'; SEQ ID NO:23) and a PM2 extension primer with a non-coding strand as template (5'TCTTGGGATGGTCATGCAAT³'; SEQ ID NO:65) may be used with the ABIPrism SnaPshot assay available from Applied Biosystems (Foster City, CA).

[046] Prior to the detection steps, template DNA containing the PM1 and PM2 mutations may optionally be amplified using known methods. Amplification and creation of a DNA template can be achieved using any method known to those of skill in the art including PCR. The term "PCR" as used herein refers to the polymerase chain reaction method of DNA amplification. As will be understood by one of ordinary skill in the art, this term also includes any and all other methods known in the art for nucleic acid amplification requiring an amplification target, at least one primer and a polymerase.

[047] For example, either PM1 template DNA or PM2 template DNA may be amplified by combining the isolated genomic DNA with an appropriate primer set for the amplification of a polynucleotide sequence containing a PM1 or PM2 mutation. Each primer set consists of a forward primer and a reverse primer, each of which can be referred to as an "amplification primer." In one embodiment of the present invention, *AHAS1* and *AHAS3* template DNAs may be amplified using a single primer set wherein a first amplification primer comprises the sequence 5' GGC GTT TGG TGT TAG GTT TGA 3' (SEQ ID NO:90) and a second amplification primer comprises the sequence 5' CGT CTG GGA ACA ACC AAA AGT 3' (SEQ ID NO:91). Alternatively, an *AHAS1* template DNA may be separately

amplified using an *AHAS1*-specific forward primer 5' GGA AAG CTC GAG GCT TTC GCT 3' (SEQ ID NO: 92) and an *AHAS1/AHAS3* reverse primer 5' ATC ACC AGC TTC ATC TCT CAG T 3' (SEQ ID NO: 93). In this embodiment, an *AHAS3* template DNA may be separately amplified using an *AHAS3*-specific forward primer (5' GGA AAG CTC GAG GCG TTT GCG 3'; SEQ ID NO: 94) and the *AHAS1/AHAS3* reverse primer (5' ATC ACC AGC TTC ATC TCT CAG T 3'; SEQ ID NO: 93).

[048] Those of ordinary skill will recognize that additional amplification primers may be prepared which are contiguous, noncontiguous or homologous primer to the amplification primers et forth above. The forward and reverse primers can also be an RNA version of any of the aforementioned amplification primers.

[049] The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof.

EXAMPLES

Example 1

Isolation of genomic DNA from a Plant

[050] The DNA extraction procedure described below (Cheung *et al.*, 1993 PCR Methods and Applications 3:69-70) can be used for both fresh and lyophilized leaf tissues. If fresh leaf tissues are used, the Phenol and chloroform/isoamyl-alcohol extraction steps can be omitted.

[051] Two 5 mm diameter leaf discs made with a paper punch or the equivalent were taken from each leaf sample and immediately placed in 320 µl of sterile extraction buffer containing 200 mM Tris-HCl (pH 8.0), 70 mM EDTA, 2 M NaCl and 20 mM sodium metabisulfite. Leaves were then ground until no visible pieces of tissue remained. Cells were lysed with addition of 80 µl of 5% sodium sarcosyl to each tube and were incubated at 60 °C for an hour. After 15 minutes of centrifugation at 13,800 RPM, the supernatant was transferred to a fresh tube and an equal volume of buffer saturated phenol was added. The contents in the tubes were mixed by inverting a few times and were spun at 13,800 RPM for 5 minutes.

[052] The aqueous phase was then transferred into a fresh tube and an equal volume of chloroform/isoamyl alcohol (24:1 v/v) was added and mixed by inverting tubes a few times and then was spun at 13,800 RPM for 5 minutes. After transferring the aqueous phase to a fresh tube, 180 µl of filter-sterilized 10 M ammonium acetate and 400 µl of isopropanol were

then added and left at room temperature for 15 minutes for DNA precipitation. After centrifuging for 15 minutes at 13,800 RPM, the supernatant was removed the pellets were rinsed once in 70% EtOH and left to air dry. The DNA pellet was resuspended in 100 µl TE buffer with 0.01 mg/ml of RNase and a 9 µl aliquot of DNA was run on 0.8% agarose to check for quantity and quality.

Example 2

DNA Amplification and Clean-Up

[053] Preliminary testing showed that the primer pair, Primer 1 (5' GGC GTT TGG TGT TAG GTT TGA 3') (SEQ ID NO:90) and Primer 2 (5' CGT CTG GGA ACA ACC AAA AGT 3') (SEQ ID NO:91) could amplify in one PCR reaction sufficient amounts of both *AHAS1* and *AHAS3* sequences for both PM1 and PM2 tests. Each PCR reaction mixture was set up in a total volume of 75 µl containing 1X PCR buffer II (Perkin Elmer), 2.5 mM MgCl₂, 200 µM of each dNTP, 400 nM each of Primer 1 and Primer 2, 100 ng of DNA (or 4 µl of extracted DNA) and 3 units of AmpliTaq® DNA polymerase (Perkin Elmer). Amplification reactions were carried out in Perkin Elmer GeneAmp 9600 or 9700 PCR systems. The PCR program included an initial denaturing step at 94 °C, followed by 30 cycles of denaturation at 94 °C for 10 seconds, annealing at 56 °C for 15 seconds, and extension at 72 °C for 30 seconds with a final extension step of 5 minutes at 72 °C. An aliquot of the PCR product was checked on 1.4 % agarose for an expected product size of 1Kb.

[054] In the clean-up step, 50 µl of each PCR product was first treated with 10 units of CIP (calf intestinal phosphatase, NEW ENGLAND BioLabs Inc.) by incubating at 37 °C for 1 hour and then deactivating the enzyme by incubating at 72 °C for 15 minutes in Perkin Elmer GeneAmp 9700 PCR systems. Subsequently, the 50 µl aliquot was purified using the QIAquick™ 96 PCR Purification Kit (QIAGEN) and eluted in 50 µl ddH₂O. Samples were then placed in a Universal Vacuum System UVS400/Speed Vac® Plus SC110A (Savant) for approximately 1 hour or until the water in the sample completely evaporated. The CIP treated and purified PCR product was resuspended in ddH₂O at a concentration of approximately 50 ng/µl and was used as DNA templates for the primer extension reactions for detecting the PM1 and PM2 mutations.

Example 3

Primer Extension PCR for Detecting PM1 and PM2 Mutations using ABI PRISM®

[055] The ABI PRISM® SNaPshot ddNTP Primer Extension Kit was used on each DNA sample and to detect both the PM1 and the PM2 single nucleotide mutations. The mutation detecting primers are as follows: PM1 extension primer: 5' CAT CTT TGA AAG TGC CAC CA 3' (SEQ ID NO:24) for detection of the PM1 mutation and PM2 extension primer: 5' CTT TGT AGA ACC GAT CTT CC 3' (SEQ ID NO:66) for detection of the PM2 mutation. Primer extension reactions were performed with 100 ng of CIP treated and purified PCR amplified templates in a total volume of 10 µl with 100 nM of the appropriate mutation primer, SNaPshot Ready Reaction Premix as indicated by the manufacturer. Thermal cycling was performed in Perkin Elmer GeneAmp 9600 or 9700 PCR systems with conditions set for 25 cycles of denaturation at 96 °C for 10 seconds, annealing at 50 °C for 5 seconds and extension at 60 °C for 30 seconds. Post-extension treatment consisted of incubating the reaction mixture for 1 hour at 37 °C with 1 unit of calf intestinal phosphatase (NEW ENGLAND BioLabs Inc.) and the enzyme was inactivated at 72 °C for 15 minutes. Samples were then prepared for loading on an ABI PRISM® 3700 DNA Analyzer by adding 1µl of each post-extension treated reaction to 10 µl of deionized formamide, denatured at 95 °C for 5 minutes and then loaded and run using a GeneScan 5 Run Module. Data was collected and viewed using the ABI PRISM® GeneScan v. 3.5.1 software.

Example 4

Detection of PM1 and PM2 Mutations in B. napus using ABI PRISM®

[056] The PM1 test using the primer PM1 involves the extension of the next nucleotide to the primer sequence with the coding strand as the template. Thus, in the wildtype plant, here a *B. napus* cv. 'Topas' plant, the observed nucleotide should be "C" corresponding to the wildtype "G" in the codon "AGT" for Serine on the coding strand. When the test is done on the mutated PM1 *B. napus* plant, the observed nucleotide should be "T" corresponding to the mutated "A" in the codon "AAT" for Asparagine on the coding strand (Figure 2). The results obtained with the ABI PRISM® method showed exactly the predicted results. A mutated PM2 *B. napus* plant that did not contain the PM1 mutation was shown to provide the same results as the wildtype 'Topas' plant in the PM1 test. Therefore, the PM1 mutation was detected accurately in *B. napus* using the ABI PRISM® primer extension methodology.

[057] Similarly, the PM2 test using the primer PM2 involves the extension of the next nucleotide to the primer sequence with the coding strand as the template. Thus, in the wildtype plant, e.g. 'Topas', the observed nucleotide should be "C" corresponding to the wildtype "G" in the codon "TGG" for Tryptophan on the coding strand. When the test was done on the mutated PM2 *B. napus* plant, the observed nucleotide should be "A" corresponding to the mutated "T" in the codon "TTG" for Leucine on the coding strand (Figure 3). The results obtained with the present method showed exactly the predicted results. A mutated PM1 *B. napus* plant that does not have the PM2 mutation was shown to provide the same results as the wildtype 'Topas' plant in the PM2 test. Therefore, the PM2 mutation was detected accurately in *B. napus* using the ABI PRISM® primer extension methodology.

Example 5

Validation of ABI PRISM® PM1 and PM2 Detection Method

[058] In order to validate the use of the present method on plant materials with a genetic background different from the one used to develop the markers and the method (the *B. napus* 'Topas' plant), the PM1 and PM2 tests were performed to detect the presence or absence of the CLEARFIELD® trait on 24 doubled haploid (DH) (*i.e.*, homozygous) canola lines. These 24 lines were divided into four classes: PM1, PM2, PM1/PM2 and WT based on the results of survival after spraying with herbicide. The codes and classification of the DH lines are summarized in Figure 4, in which "GH Rating" means greenhouse rating on mortality: 0 means all plants survive after spraying and 85% means 85% of the plants died after spraying. Also included in Figure 4 are the three controls used in the validation tests: PM1, PM2 and WT, all from the *B. napus* 'Topas' var. used in Examples 2 through 4 for development of the PM1/PM2 assay. The amplification of the templates and the mutation tests were repeated three times for each DH line from Advanta Seeds and twice for the three control samples.

[059] The results of the PM1 and PM2 mutation tests are summarized in Figure 5. The plant number in Figure 5 corresponds to the plant number in Figure 4. Additionally, the peaks related to the mutations are in bold and in italics while the peaks that are not always present or present in various amounts in all the three replicates are in brackets. The "Expected Results" column reflects those results that are expected assuming that the amplification reaction using the primer pair *AHAS1/AHAS3* amplification primer of SEQ ID NO: 90 and the *AHAS1/AHAS3* amplification primer of SEQ ID NO: 91 amplified similar

amounts of both *AHAS1* and *AHAS3* sequences and that the PM1 extension primers will anneal also to the *AHAS3* sequence and the PM2 extension primers will anneal also to the *AHAS1* sequence.

[060] As shown in Figure 5, the observed results for both the PM1 and PM2 mutation tests agreed with the expected results for all six plants in the PM1/PM2 class. With the PM1 class, all six plants showed the PM1 mutation (as "T"). All of the wild-type plants showed the absence of either mutation. Therefore, with all three classes of plants, the present invention can correctly predict the presence or absence of the PM1 and PM2 mutations.

[061] The results for the PM2 class were more complicated. All the six plants of the PM2 classes were expected to have the PM2 mutation (i.e. an "A" with the PM2 mutation test). In fact, all the six plants did detect an "A" with the test throughout the three replicates. The PM2 class was expected to have the wild-type "C" for the PM1 mutation test. However, in the observed results, only plant #40 showed the wild-type "C", while each of the other five plants consistently showed a "T" for the PM1 mutation test, indicating the unexpected presence of the of the PM1 mutation. The control lines gave the expected results.

[062] It is believed that the discrepancy in the expected and actual results regarding the plants classified as containing only the PM2 mutation is due to misclassification under the herbicide spraying test and that this discrepancy reflects the superiority of the present invention. One advantage of using the present invention to identify the presence or the absence of PM1 or PM2 mutations over the herbicide spraying test is that the present invention can unequivocally tell whether the mutations are present in the genetic materials of the tested plants. Hence, the invention described herein presents a more reliable test which will not be influenced by other environmental factors.

[063] Using the present invention, one can easily tell apart the wild type plants from those with only the PM1 mutation and also differentiate between plants with only PM1 or PM2 mutations and those with both PM1 and PM2 mutations, which are particularly difficult to distinguish using the spraying test. With the prior art herbicide spraying test, a statistical number of plants of the same line need to be grown and sprayed to obtain meaningful results while with the present invention, fewer plants from the same line need to be tested. Since the methods of the present invention only require very small amount of leaf materials per line, another advantage of these methods is that they can be performed when the plants are very young, for example at the cotyledon stage. This advantage translates into savings in growth space and other costs.

Example 6

Detection of PM1 and PM2 Mutations in B. napus using PYROSEQUENCING PSQ™ 96

[064] A second method to allow high throughput detection of the presence or absence of "PM1" and "PM2" mutations in *B. napus* was designed, the method comprising four steps:

1. Isolation of genomic DNA
2. Separation of *AHAS1* and *AHAS3* DNA template preparations by PCR with an *AHAS1*-specific forward primer paired with a biotinylated *AHAS1/AHAS3* reverse primer for *AHAS1* and an *AHAS3*-specific forward oligonucleotide primer paired with the same biotinylated *AHAS1/AHAS3* reverse primer for *AHAS3*
3. Isolation of single stranded DNA templates
4. PYROSEQUENCING™ reactions with PM1 sequencing primer for detecting the "PM1" mutation and PM2 sequencing primer for detecting the "PM2" mutations.

DNA Isolation

[065] The procedure set forth in Example 1 was used to isolate DNA from plants for analysis using the PYROSEQUENCING™ method.

DNA amplification

[066] For detection of the PM1 and PM2 mutations using the PYROSEQUENCING™ method, the best results were obtained when *AHAS1* and *AHAS3* sequences were separately amplified as templates. Therefore, two amplification reactions were first performed using different forward primers, *AHAS1*-specific forward primer for *AHAS1* (5' GGA AAG CTC GAG GCT TTC GCT 3'; SEQ ID NO:92) and *AHAS3*-specific forward primer for *ALSS3* (5' GGA AAG CTC GAG GCG TTT GCG 3'; SEQ ID NO: 94) but pairing with the same biotinylated reverse primer, *AHAS1/AHAS3* reverse primer (5' ATC ACC AGC TTC ATC TCT CAG T 3'; SEQ ID NO:93). Each PCR reaction was set up in a total volume of 30 µl containing 1X PCR buffer II (Applied Biosystems, Foster City, CA), 2.5 mM MgCl₂, 200 µM of each dNTP, 300 nM each of an *AHAS1*-specific forward primer and *AHAS1/AHAS3* reverse primer for *AHAS1* and an *AHAS3*-specific forward primer and *AHAS1/AHAS3* reverse primer for *AHAS3*, 5 ng of DNA and 1.25 units of AmpliTaq® Gold DNA polymerase (Applied Biosystems, Foster City, CA). Amplification reactions were carried out in Applied Biosystems GeneAmp 9600® or GeneAmp 9700® PCR systems. The PCR program includes an initial denaturing step at 94°C for 10 minutes, followed by 45 cycles of denaturation at 94°C for 10 seconds, annealing at 56°C for 15 seconds, and

extension at 72°C for 30 seconds with a final extension step of 10 minutes at 72°C. An aliquot of each PCR product was checked on 1% agarose for an expected product size of 1Kb.

Single strand template isolation and annealing of sequencing primers for detection of "PM1" and "PM2" mutations

[067] PCR amplified products were immobilized by mixing 25 µl of the PCR product with 150 ng of Dynabeads® M-280 Streptavidin (DynaL AS, Oslo, Norway) and 25 µl of 2X Binding-Washing buffer II pH 7.6 (PYROSEQUENCING™) and were incubated on an agitator at 65° for 30 minutes. Using the PSQ 96 Sample Prep Tool, the beads carrying the biotinylated templates were then transferred and released into a PSQ 96 Plate containing 50 µl of 0.5 M NaOH per well and left to soak with gentle agitation for 1 minute. The beads now carrying the isolated biotinylated non-coding strands were then transferred into a second PSQ™ 96 Plate for a wash in 100 µl of 1X annealing buffer (PYROSEQUENCING™). Finally, annealing of the sequencing primers was done by transferring the beads into a third PSQ 96 Plate containing 44 µl of 1X annealing buffer (PYROSEQUENCING™) and either 10 pmol of PM1 sequencing primer (5' GTG TTA CCG ATG ATC C 3'; SEQ ID NO: 95) or 10 pmol of PM2 sequencing primer (5' GGG ATG GTC ATG CAA T 3'; SEQ ID NO: 96) for assaying the PM1 and PM2 mutations respectively. This third plate was then incubated at 94°C for 3 minutes and allowed to cool to room temperature for 5 to 10 minutes.

SNP detection using the PYROSEQUENCING (PSQ™ 96) system

[068] The third PSQ 96 Plate containing PM1 or PM2 sequencing primers annealed to the non-coding biotinylated strands from each PCR product was loaded onto the PSQ™ 96 system and the pyrosequencing run was carried out using the PSQ™ 96 Instrument Control module from the PSQ™ 96 SNP Software (version 1.2 AQ). The PSQ™ 96 SNP Entry module was used to enter the orders of dispensing nucleotides for both PM1 and PM2 detection (CTAGCTGTG for "PM1" detection and CTGCAGATC for "PM2" detection) while the PSQ™ 96 Evaluation module was used for viewing the results of pyrosequencing.

[069] The choice of the non-coding sequence as the template and the specific sequencing primers combinations for the "PM1" and "PM2" assay was the result of optimization of the process to produce unambiguous pyrograms that could infer the presence or absence of the mutations and whether they are present in the homozygous or heterozygous state.

Results of "PM1" and "PM2" tests using Pyrosequencing™

[070] Using the pyrosequencing technology platform for the "PM1" and "PM2" tests requires that the *AHAS1* and *AHAS3* sequences around the mutations to be amplified separately by specific PCR reactions. In the pyrosequencing technology, the incorporation of each nucleotide with the release of pyrophosphate during the primer extension reaction is coupled to the sulfurylase/luciferase system, which gives light signals proportional to the number of nucleotides incorporated at each elongation step. The results of the pyrosequencing reaction indicate the identity of the nucleotide sequences around the polymorphic site from which the nucleotide at the polymorphic site can be read. With the PM1 test, both *B. napus* 'Topas' and the *B. napus* 'PM2' line have the wildtype *AHAS1* sequence and the sequence extended from the PM1 sequencing primer is CAAGTGGTGG (SEQ ID NO:97); while for the mutant PM1 line, the extended sequence is CAAATGGTGG (SEQ ID NO:98) indicating the G→A PM1 mutation on the coding strand. With the PM2 test, both 'Topas' and the 'PM1' line have wildtype *AHAS3* sequence and the sequence extended from the PM2 sequencing primer is GGGAAGATC (SEQ ID NO:99); while for the mutant PM2 line, the extended sequence is TGGAAGATC (SEQ ID NO:100) indicating the G→T PM2 mutation on the coding strand. Thus both PM1 and PM2 mutations were detected accurately using the PYROSEQUENCING™ technology.

[071] Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in are hereby incorporated by reference in their entireties. It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

CLAIMS

1. A method of assaying a plant for imidazolinone herbicide resistance conferred by the combination of a PM1 mutation of a *B. napus* *AHAS1* gene and a PM2 mutation of a *B. napus* *AHAS3* gene, the method comprising the steps of:

- a) isolating genomic DNA from the plant;
- b) determining the presence or absence of the PM1 mutation in the DNA; and
- c) determining the presence or absence of the PM2 mutation in the DNA,

wherein the presence of the PM1 mutation and the PM2 mutation is indicative of commercially relevant imidazolinone tolerance in the plant.

2. The method of claim 1, wherein the plant is a *Brassica* species.

3. The method of claim 2, wherein the *Brassica* species is selected from the group consisting of *B. napus*, *B. campestris/rapa*, and *B. juncea*.

4. The method of claim 1, further comprising the step of amplifying the isolated DNA prior to determining the presence or absence of the PM1 and PM2 mutations.

5. The method of claim 1, wherein the determining steps are performed using a primer extension-based single nucleotide polymorphism detection method.

6. A PM1 primer extension oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39; SEQ ID NO:40; SEQ ID NO:41; SEQ ID NO:42 and SEQ ID NO:95.

7. A PM1 oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:45 and SEQ ID NO:46.

8. A PM2 primer extension oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58; SEQ ID NO:59; SEQ ID NO:60; SEQ ID NO:61; SEQ ID NO:62; SEQ ID NO:63; SEQ ID NO:64; SEQ ID NO:65; SEQ ID NO:66; SEQ ID NO:67; SEQ ID NO:68; SEQ ID NO:69; SEQ ID NO:70; SEQ ID NO:71; SEQ ID NO:72; SEQ ID NO:73; SEQ ID NO:74; SEQ ID NO:75; SEQ ID NO:76; SEQ ID NO:77; SEQ ID NO:78; SEQ ID NO:79; SEQ ID NO:80; SEQ ID NO:81; SEQ ID NO:82; SEQ ID NO:83; SEQ ID NO:84 and SEQ ID NO:96.

9. A PM2 oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:87 and SEQ ID NO:88.

10. An oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:85 and SEQ ID NO:86.

11. An amplification oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO:90; SEQ ID NO:91; SEQ ID NO:92; SEQ ID NO:93; and SEQ ID NO:94.

**PM1-AHAS1 nucleotide sequence (SEQ ID NO: 1) and
translated amino acid sequence (SEQ ID NO: 101)**

1 TCATCATCTCTCTCTCTCAAAACCATGGCGGCGCAACATCGTCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAATCCCCTCTACCCATTTC
M A A A T S S S P I S L T A K P S S K S P L P I S

101 GATTCTCCCTTCCTTCTCTTAACCCACAGAAAGACTCCTCCCGTCTCCACCGTCTCTCGCCATCTCCGCCGTTCTCAACTACCCGTCATGTCGC
R F S L P F S L T P Q K D S S R L H R P L A I S A V L N S P V N V A

201 ACCTCTTCCCTGAAAAACCGACAAGAACAAGACTTTCTGCTCCCGCTACGCTCCGACGAGCCCGCAAGGGTGCTGATATCCTCGTCGAAGCCCTC
P P S P E K T D K N K T F V S R Y A P D E P R K G A D I L V E A L

301 GAGCGTCAAGCGCTCGAAACCGTCTTCTGCTTATCCCGAGGTGCTTCCATGGAGATCCACCAAGCCTTGACTCGCTCCTCCACCATCCGTAACGTCCTTC
E R Q G V E T V F A Y P G G A S M E I H Q A L T R S S T I R N V L

401 CCCGTCACGAACAAGGAGGAGTCTTCGCGCGCGAGGTTACGCTCGTTCCTCCGCAACCGGGAATCTGCATAGCCACTTCGGGTCCCGGAGCTACCAA
P R H E Q G G V F A A E G Y A R S S G K P G I C I A T S G P G A T N

501 CCTCGTCAGCGGGTTAGCAGACCGATGCTTGACAGTGTCTCTTGTGCCATTACAGGACAGTCCCTCGCGGATGATCGGTACTGACGCTTCCAA
L V S G L A D A M L D S V P L V A I T G Q V P R R M I G T D A F Q

601 GAGACCAATCGTTGAGGTAACGAGGTCTATTACGAACATAACTATCTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAGCATTCTTTC
E T P I V E V T R S I T K H N Y L V M D V D D I P R I V Q E A F F

701 TAGCTACTTCCGCTAGACCCGACCGGTTTGGTTGATGTTCTAAGGATATTCAGCAGCAGCTTGGGATTCCTAACTGGGATCAACCTATGCGCTTGCC
L A T S G R P G P V L V D V P K D I Q Q Q L A I P N W D Q P M R L P

801 TGGCTACATGCTAGGTTGCTCAGCCWCCGAAGTTTCTCAGTTAGGTCAGATCGTTAGGTTGATCTCGGAGTCTAAGAGGCTGTTTGTACGTTGGT
G Y M S R L P Q X P E V S Q L G Q I V R L I S E S K R P V L Y V G

901 GGTGGAAGCTTGAACGAGTGAAGAAGTGGGAGATTTCTCGAGCTTACTGGGATCCCTGTTGCGAGTACGTTGATGGGGCTTGGCTCTTATCCTTGT
G G S L N S S E E L G R F V E L T G I P V A S T L M G L G S Y P C

1001 ACGATGAGTTGTCCTGCAGATGCTTGGCATGCACGGGACTGTGTATGCTAAGTACGCTGTGGAGCATAGTGATTGTTGCTGGCGTTTGGTGTAGGTT
N D E L S L Q M L G M H G T V Y A N Y A V E H S D L L A F G V R F

1101 TGATGACCGTGTACGGGAAAGCTCGAGGCTTTCGCTAGCAGGGCTAAATTTGTGCACATAGACATTGATTTCTGCTGAGATTGGGAAGAATAAGACACCT
D D R V T G K L E A F A S R A K I V H I D I D S A E I G K N K T P

1201 CACGTGCTGTGTGTGGTGATGTAAGCTTGGCTTTCGAAGGGATGAACAAGGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTCGGTGTGGA
H V S V C G D V K L A L Q G M N K V L E N R A E E L K L D F G V W

1301 GGAGTGAGTTGAGCGAGCAGAAACAGAAGTCCCTTTGAGCTTCAAAACGTTTGGAGAAGCCATTCTCCGAGTACCGGATTCAGATCCTCGACGAGCT
R S E L S E Q K Q K F P L S F K T F G E A I P P Q Y A I Q I L D E L

1401 AACCGAAGGGAAGGCAATTATCAGTACTGGTGTGGACAGCGTCAGATGTGGCGGCGCAGTTTACAAGTACAGGAAGCCGAGACAGTGGCTGTGCTCA
T E G K A I I S T G V G Q R Q M W A A Q F Y K Y R K P R Q W L S S

1501 TCAGGCTCCGAGCTATGGGTTTGGACTTCTGCTGCGATTGGAGCGTCTGTGGCGAACCTGATGCGATTGTTGTGGATATTGACGGTGATGGAAGCT
S G L G A M G F G L P A A I G A S V A N P D A I V V D I D G D G S

1601 TCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTTCTGTGAAGATACTCTTGTAAACAACCAGCATCTTGGGATGGTCATGCAATG
F I M N V Q E L A T I R V E N L P V K I L L L N N Q H L G M V M Q W

1701 GGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGAGAACGAGATCTTCCCTAACATGCTGCAGTTTGCAGGAGCT
E D R F Y K A N R A H T Y L G D P A R E N E I F P N M L Q F A G A

1801 TGCGGATTCAGCTGCGAGAGTGACGAAGAAGAAGAAGTCCGAGAAGTATTAGACAATGCTGGATACCAAGGACCACCTGTTGGATGTGATAT
C G I P A A R V T K K E E L R E A I Q T M L D T P G P Y L L D V I

1901 GTCCGACCAAGAATGTGTTACCGATGATCCCAATGGTGGCACTTTCAGAGTGAATAACAGAAGGGGATGGTCGCACTAAGTACTGAGAGATTMA
C P H Q E H V L P M I P N G G T F K D V I T E G D G R T K Y

2001 GCTGGTGATCGATCATATGTTAAAGACTTAGTTTCAGTTTCAGTTCTTGTGTGGTAATTTGGGTTTGTGAGTTGTTGT

FIG. 1A

PM2-AHAS3 nucleotide sequence (SEQ ID NO:2) and translated amino acid sequence (SEQ ID NO: 102)

1 TTCATCATMTCTCTCATTTCTCTCTCTCTCATCTAACCATGGCGCGGCAACATCGTCTTCTCCGATCTCCTTAACCGTAAACCTTCTTCCAAAT
M A A A T S S S P I S L T A K P S S K

101 CCCCTCTACCCATTTCAGATTCTCCCTTCCCTTCTCCTTAACCCACAGAAACCTCTCCCGTCTCCACCGTCCACTCGCCATCTCCGCGGTCTCAA
S P L P I S R F S L P F S L T P Q K P S S R L H R P L A I S A V L N

201 CTCACCCGTCAATGTCCGACCTGAAAAACCGACAAGATCAAGACTTTCATCTCCCGCTACGCTCCCGACGAGCCCGCAAGGGTGCTGATATCCTCGTG
S P V N V A P E K T D K I K T F I S R Y A P D E P R K G A D I L V

301 GAAGCCCTCGAGCGTCAAGGCGTCAAAACCGTCTTCGCTTATCCCGGAGGTGCCTCCATGGAGATCCACCAAGCCTTGACTCGCTCCTCCACCATCCGTA
E A L E R Q G V E T V F A Y P G G A S M E I H Q A L T R S S T I R

401 ACGTCTCCCCCGTACGAAACAAGGAGGAGTCTTCGCGCCGAGGGTTACGCTCGTTCCTCCGGCAAACCGGGAATCTGCATAGCCACTTCGGGTCCCGG
N V L P R H E Q G G V F A A E G Y A R S S G K P G I C I A T S G P G

501 AGCTACCAACCTCGTCAGCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGTCGCCATCAGGACAGGTCCCTCGCCGATGATCGGTACTGAC
A T N L V S G L A D A M L D S V P L V A I T G Q V P R R M I G T D

601 GCGTTCGAAGAGACGCCAATCGTTGAGGTAAACGAGGTCTATTACGAAACATAACTATCTGGTGATGGATGTGTGATGACATACCTAGGATCGTTCAAGAAG
A F Q E T P I V E V T R S I T K H N Y L V M D V D D I P R I V Q E

701 CATCTTCTTAGTACTTCCGGTAGACCCGACCGGTTTGGTTGATGTTCTTAAGGATATTACGACGAGCTTGCATTCCTAACTGGGATCAACCTAT
A F F L A T S G R P G P V L V D V P K D I Q Q Q L A I P N W D Q P M

801 GCGCTTGCTGGCTACATGCTTAGGCTGCCTCAGCCACCGGAAGTTTCTCAGTTAGGCCAGATCGTTAGGTGATCTCGGAGTCTAAGAGCGCTGTTTTG
R L P G Y M S R L P Q P P E V S Q L G Q I V R L I S E S K R P V L

901 TACGTTGGTGGTGAAGCTTGAACCTCGAGTGAAGAACTGGGGAGATTGTCGAGCTTACTGGGATCCCTGTGTGCGAGTACGCTGATGGGGCTTGCTCTT
Y V G G G S L N S S E E L G R F V E L T G I P V A S T L M G L G S

1001 ATCCTTGTAACGATGAGTTGTCCTGCAGATGCTTGGCATGACCGGACTGTGTATGCTAACTACGCTGTGGAGCATAGTGATTTGTTGCTGGCCTTTGG
Y P C N D E L S L Q M L G M H G T V Y A N Y A V E H S D L L L A F G

1101 TGTTAGGTTTGATGACCGTGTACGGGAAAGCTCGAGCGGCTTTCGAGCAGGGCTAAGATTGTCACATAGACATTGATTCTGCTGAGATTGGGAAGAAT
V R F D D R V T G K L E A F A S R A K I V H I D I D S A E I G K N

1201 AAGACACCTCACGTGTCTGTGTGTGGTGATTAAGCTGGCTTTGCAAGGGATGAACAAGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTTCG
K T P H V S V C G D V K L A L Q G M N K V L E N R A E E L K L D F

1301 GTGTTTGGAGGAGTGAGTTGAGCGAGCAGAAACAGAAGTTCCTGTTGAGCTTCAAAACGTTTGGAGAAGCCATTCTCCGAGTACCGGATTCAGGTCTCT
G V W R S E L S E Q K Q K F P L S F K T F G E A I P P Q Y A I Q V L

1401 AGACGAGCTAACCCAAGGGAAGGCAATTATCAGNACTGGTGTGGACAGCATCAGATGTGGCGCGCAGTTTACAAGTACAGGAAGCCGAGGCAAGTGG
D E L T Q G K A I I X T G V G Q H Q M W A A Q F Y K Y R K P R Q W

1501 CTGTCGTCTCAGGACTCGGAGCATGCGGTTTCGGACTTCTGCTGCGATTGGAGCGTCTGTGGCGAACCTTGATGCGATTGTTGTGGACATTGACGGTG
L S S S G L G A M G F G L P A A I G A S V A N P D A I V V D I D G

1601 ATGGAAGCTTCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTTCTGTGAAGATACTCTTGTAAACAACCGACATCTTGGGATGGT
D G S F I M N V Q E L A T I R V E N L P V K I L L L N N Q H L G M V

1701 CATGCAATTGGAAGATCGGTCTTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGGAGAACGAGATCTTCCCTAACATGTGCAAGTTT
M Q L E D R F Y K A N R A H T Y L G D P A R E N E I F P N M L Q F

1801 GCAGGAGCTTCCGGGATTCAGCTGCGAGAGTGACGAAGAAGAAGAACTCCGAGAAGCTATTGAGACAATGCTGGATACCTGGACCTACCTGTTGG
A G A C G I P A A R V T K K E E L R E A I Q T M L D T P G P Y L L

1901 ATGCCATCTGTCCGACCAAGAACATGTGTTACCGATGATCCCAAGTGGTGGCACTTTCAAAGATGTAATAACCGAAGGGGATGGTGCCTAAGTACTG
D A I C P H Q E H V L P M I P S G G T F K D V I T E G D G R T K Y

2001 AGAGATGAAGCTGGTGATCCATCGTATGGTAAAGACTTAGTTTCAGTTTTCAGTTTCTTTTGTGTGGAATTTGGGTTTGTGAGTTGTTGTTTGTCTT

2101 TGGTTTGTTCCKNAC

FIG. 1B

**T-AHAS1 nucleotide sequence (SEQ ID NO:3) and
translated amino acid sequence (SEQ ID NO: 103)**

1 TCATCATCTCTCTCTCTCTAACCATTGGCGGGCGGCAACATCGTCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAATCCCTCTACCCATTCCA
M A A A T S S S P I S L T A K P S S K S P L P I S
101 GATTCTCCCTTCCCTTCTCTTAAACCCACAGAAAGACTCCTCCCGTCTCCACCGTCTCTCGCCATCTCCGCGTCTCAACTCACCCTCAATGTCGC
R F S L P F S L T P Q K D S S R L H R P L A I S A V L N S P V N V A
201 ACCTCCTTCCCTGAAAAACCGACAAGAACAAGACTTTCTGCTCCCGCTACGCTCCCGACGAGCCCGCAAGGGTGCTGATATCTCGTCGAAGCCCTC
P P S P E K T D K N K T F V S R Y A P D E P R K G A D I L V E A L
301 GAGCGTCAAGGCGTCGAAACCGTCTTGCTTATCCCGAGGTGCTTCCATGGAGATCCACCAAGCCTTGACTCGCTCTCCACCATCCGTAACGCTCTTC
E R Q G V E T V F A Y P G G A S M E I H Q A L T R S S T I R N V L
401 CCCGTACGAACAAGGAGGAGTCTTCGCCGCGGAGGGTTACGCTCGTTCCTCCGCAACCGGGAATCTGCATAGCCACTTCGGGTCCCGAGCTACCAA
P R H E Q G G V F A A E G Y A R S S G K P G I C I A T S G P G A T N
501 CCTCGTCAGCGGGTTAGCAGACGCGATGCTTGACAGTGTCTCTTGTGCGCATTACAGGACAGGTCCCTCGCCGGATGATCGGTACTGACGCTTCCAA
L V S G L A D A M L D S V P L V A I T G Q V P R R M I G T D A F Q
601 GAGACACCAATCGTTGAGGTAACGAGGTCTATTACGAAACATAACTATTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAGCTTTCTTTC
E T P I V E V T R S I T K H N Y L V M D V D D I P R I V Q E A F F
701 TAGCTACTTCCGCTAGACCCGACCGGTTTGGTGTGATGTTCTAAGGATATTACGACGAGCTTGGATTCTTAAGGGATCAACCTATGCGCTTACC
L A T S G R P G P V L V D V P K D I Q Q Q L A I P N W D Q P M R L P
801 TGGCTACATGTCTAGGTGCTCAGCCTCCGGAAGTTTCTCAGTTAGGTGATGCTTGGATTGATCTCGGAGTCTAAGAGCCCTGTTTGTACGTTGGT
G Y M S R L P Q P P E V S Q L G Q I V R L I S E S K R P V L Y V G
901 GGTGGAAGCTTGAAGTGAAGAACTGGGAGATTGTCGAGCTTACTGGGATCCCGTTGCCGAGTACTTGTATGGGCTTGGCTCTTATCCTTGTGA
G G S L N S S E E L G R F V E L T G I P V A S T L M G L G S Y P C
1001 ACATGAGTTGCTTGCATGCTTGGCATGCACGGGACTGTGTATGCTAAGTACGCTGTGGAGCATAGTGATTGTTGCTGGCGTTGGTGTAGGTT
N D E L S L Q M L G M H G T V Y A N Y A V E H S D L L L A F G V R F
1101 TGATACCGTGTACCGGAAAGCTCGAGGCTTTTCGCTAGCAGGGCTAAAATTGTGCACATAGACATTGATTCTGCTGAGATTGGGAAGAATAAGACACCT
D D R V T G K L E A F A S R A K I V H I D I D S A E I G K N K T P
1201 CACGTCTCTGTGTGTGTGATGTAAGCTGGCTTTGCAAGGGATGAACAAGTTCTTGAGAACCGGGCGGAGGAGCTCAAGCTTGATTTCGGTGTGGA
H V S V C G D V K L A L Q G M N K V L E N R A E E L K L D F G V W
1301 GGAGTGAGTTGAGCGAGCAGAAACAGAAGTTCCCTTTGAGCTTCAAACGTTTGAGAAGCCATTCTCCGAGTACCGGATTGATCTCTGACGAGCT
R S E L S E Q K Q K F P L S F K T F G E A I P P Q Y A I Q I L D E L
1401 AACCGAAGGGAAGGCAATTATCAGTACTGGTGTGACAGCATCAGATGTGGCGGCGCAGTTTACAAGTACAGGAAGCCGAGACAGTGGCTGTGCTCA
T E G K A I I S T G V G Q H Q M W A A Q F Y K Y R K P R Q W L S S
1501 TCAGGCTCGGAGCTATGGGTTTGGACTTCTGCTGCGGATTGGAGCGTCTGTGGCGAACCTGATGCGATTGTTGTGGATATTGACGGTGATGGAAGCT
S G L G A M G F G L P A A I G A S V A N P D A I V V D I D G D G S
1601 TCATAATGAACGTTCAAGAGCTGGCCACAATCCGTGTAGAGAATCTCTGTGAAGATACTCTGTGTAACAACAGCATCTTGGGATGGTCATGCAATG
F I M N V Q E L A T I R V E N L P V K I L L L N N Q H L G M V M Q W
1701 GGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGACCCGGCAAGGAGAACGAGATCTCCCTAACATGCTGCAGTTTGCAGGAGCT
E D R F Y K A N R A H T Y L G D P A R E N E I F P N M L Q F A G A
1801 TGCGGGATTCAGCTGCGAGAGTGACGAAGAAGAAGAACTCCGAGAAGCTATTACAGCAATGCTGGATACACAGGACCATACTGTTGGATGTGATAT
C G I P A A R V T K K E E L R E A I Q T M L D T P G P Y L L D V I
1901 GTCCGACCAAGAACATGTGTTACCGATGATCCCAAGTGGTGGCACTTTCAAGATGTAATAACAGAAGGGGATGGTCGCACTAAGTACTGAGAGATGAA
C P H Q E H V L P M I P S G G T F K D V I T E G D G R T K Y
2001 GCTGGTATCGATCATATGGTAAAGACTTAGTTTCAGTTTCCAGTTTCTTTTGTGGTAATTTGGGTTTGTGAGTTGTTGT

FIG. 1C

**T-AHAS3 (SEQ ID NO: 4) nucleotide sequence and
translated amino acid sequence (SEQ ID NO: 104)**

```

1  TTMACATCTCTCTCATTCCTCTCTCCCTCATCTAACCATGGCGGCGGCAACATCGCCTTCTCCGATCTCCTTAACCGCTAAACCTTCTTCCAAAT
    M A A A T S P S P I S L T A K P S S K
101 CCCCTCTACCCATTTCAGATTCTCCCTTCTCCTTAACCCACAGAAACCTCTCCCGTCTCCACCGTCCACTCGCCATCTCCGCCGTCTCAA
    S P L P I S R F S L P F S L T P Q K P S S R L H R P L A I S A V L N
201 CTCACCGTCAATGTGCGACCTGAAAAACGACAAGATCAAGACTTTCATCTCCCGTACGCTCCCGACGAGCCCGCAAGGGTGCTGATATCTCGTG
    S P V N V A P E K T D K I K T F I S R Y A P D E P R K G A D I L V
301 GAAGCCCTCGAGCGTCAAGGCGTCAAAACCGTCTTCGCTTATCCCGAGGTGCCATGGAGATCCACCAAGCCTTGACTCGCTCTCCACCATCCGTA
    E A L E R Q G V E T V F A Y P G G A S M E I H Q A L T R S S T I R
401 ACGTCTCCCGCTCAGCAACAGGAGGAGTCTTCGCGCGCGAGGGTTACGCTCGTCTCCGGCAACCGGGAATTCGATAGCCACTTCGGGTCCCGG
    N V L P R H E Q G G V F A A E G Y A R S S G K P G I C I A T S G P G
501 AGCTACCAACCTCGTCAGCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGTCGCCATCACAGGACAGGTCCCTCGCGGATGATCGGTACTGAC
    A T N L V S G L A D A M L D S V P L V A I T G Q V P R R M I G T D
601 GCGTTCACAGAGACGCAATCGTTGAGGTAAAGAGTCTATTACGAACATAACTATCTGGTGATGGATGTTGATGACATACCTAGGATCGTTCAAGAAG
    A F Q E T P I V E V T R S I T K H N Y L V M D V D D I P R I V Q E
701 CATCTCTTCTAGTACTTCCGGTAGACCCGACCGGTTTGGTGTGATGTTCTTAAGGATATTACAGCAGAGCTTGGGATTCTTAAGTGGGATCAACCTAT
    A F F L A T S G R P G P V L V D V P K D I Q Q Q L A I P N W D Q P M
801 GCGCTTGCTGGCTACATGTCTAGGCTGCCTCAGCCACCGGAAGTTTCTCAGTTAGGCCAGATCGTTAGGTTGATCTCGGAGTCTAAGAGCGCTGTTTG
    R L P G Y M S R L P Q P P E V S Q L G Q I V R L I S E S K R P V L
901 TACGTTGGTGGTGAAGCTTGAAGTCAAGTCAAGGAGTGGGAGATTTGTCGAGCTTACTGGGATCCCTGTTGCGAGTACGTTGATGGGGCTTGGCTCTT
    Y V G G G S L N S S E E L G R F V E L T G I P V A S T L M G L G S
1001 ATCCTTGTAAACGATGAGTTGTCCTGCAGATGCTTGGCATGCACGGGACTGTGTATGCTAACTACGCTGTGGAGCATAGTGATTTGTTGCTGGCCTTTGG
    Y P C N D E L S L Q M L G M H G T V Y A N Y A V E H S D L L L A F G
1101 TGTTAGGTTTGATGACCGTGTACCGGAAAGCTCGAGGCGTTTTCGAGCAGGGCTAAGATTGTGCACATAGACATTGATCTGCTGAGATTGGGAAGAAT
    V R F D D R V T G K L E A F A S R A K I V H I D I D S A E I G K N
1201 AAGACACCTCAGTGTCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
    K T P H V S V C G D V K L A L Q G M N K V L E N R A E E L K L D F
1301 GTGTTTGGAGGAGTGAGTTGAGCGAGCAGAAACAGAAAGTTCCTCGGTTGAGCTTCAAAACGTTTGGAGAAGCCATTCTCCGAGTACCGGATTGAGTCTT
    G V W R S E L S E Q K Q K F P L S F K T F G E A I P P Q Y A I Q V L
1401 AGACGAGCTAACCAAGGAAGGCAATTATCAGTACTGGTGTGGACAGCATCAGATGTGGCGGCGCAGTTTACAAGTACAGGAAGCCGAGGCGAGTGG
    D E L T Q G K A I I S T G V G Q H Q M W A A Q F Y K Y R K P R Q W
1501 CTGTCGCTCTCAGGACTCGGAGCTATGGGTTTCGGACTTCTGCTGCGATTGGAGCGTCTGTGGCGAACCCTGATGCGATTGTTGTGGACATTGACGGTG
    L S S S G L G A M G F G L P A A I G A S V A N P D A I V V D I D G
1601 ATGGAAGCTTCATAATGAAGCTTCAAGAGCTGGCCACAATCCGTGTAGAGAACTTCTCTGTGAAGATACTCTTGTAAACAACCAGCATCTTGGGATGGT
    D G S F I M N V Q E L A T I R V E N L P V K I L L L N N Q H L G M V
1701 CATGCAATGGGAAGATCGGTTCTACAAAGCTAACAGAGCTCACACTTATCTCGGGGACCCGGCAAGGAGAACGAGATCTTCCTAACATGCTGCAGTTT
    M Q W E D R F Y K A N R A H T Y L G D P A R E N E I F P N M L Q F
1801 GCAGGAGCTTGCAGGATTCAGCTGCGAGAGTGACGAAGAAGAAGAACTCCGAGAAGTATTCAGACAATGCTGGATACCTGGACCGTACCTGTTGG
    A G A C G I P A A R V T K K E E L R E A I Q T M L D T P G P Y L L
1901 ATGTCATCTGTCCGACCAAGAACATGTGTTACCGATGATCCCAAGTGGTGGCACTTTCGAAGATGTAATAACCGAAGGGATGGTGGCACTAAGTACTG
    D V I C P H Q E H V L P M I P S G G T F E D V I T E G D G R T K Y
2001 AGAGATGAAGCTGGTGATCCATCATATGGTAAAAGACTTAGTTTCAGTTTACAGTTTCTTTTGTGTGTAATTTGGGTTTGTAGTTGTTGTTCTGCTTT
    2101 TGGTTTGTCCCKWAC

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FIG. 1D

List of oligonucleotides suitable for detection of the PM1 and PM2 mutations. All oligonucleotides are in 5'-3' orientation.

SEQ ID NO.	AHAS1-PM1 forward	SEQ ID NO.	AHAS1-PM1 reverse	SEQ ID NO.	AHAS3-PM2 forward	SEQ ID NO.	AHAS3-PM2 reverse
5	TTATCTCGGGACCGGCAA	24	CATCTTTGAAGTGCCACCA	47	CTCAGGACTCGGAGCTATGG	66	CTTTGTAGAACCGATCTCC
6	GACCGGCAAGGGAGAACGA	25	TCTGTATTATCATCTTTGAA	48	GGAGCTATGGGTTTCGGACT	67	GCTCTGTAGCTTTGTAGAA
7	GGGAGAACGAGATCTCCCT	26	ACCATCCCTTCTGTATTA	49	GTTTCGGACTTCTGCTGG	68	ATAAGTGTAGCTCTGTAG
8	GATCTTCCCTAACATGCTGC	27	ACTTAGTGCACCATCCCT	50	TCTGCTGGATTTGGAGCGT	69	GGTCCCGAGATAAGTGTGA
9	AACATGCTGCAGTTTGCAGG	28	ATCTCTCAGTACTTGTGCG	51	ATTGGAGCTCTGTGGCGAA	70	TCCCTTCCCGGGTCCCGAG
10	AGTTTGCAGGAGCTTGGGG	29	CACCAAGCTTCTCTCAGT	52	CTGTGGGAACTTGTGCGG	71	GATCTCTCTCCCTTGGCG
11	AGCTTGCAGGATTCAGCTG	30	TATGATCGATCACCAGCTTC	53	CCCTGATGGATTTGTGCG	72	TGTTAGGAAAGATCTCGTTC
12	ATTCCAGCTGGAGAGTGAC	31	TCCTTTTACCCTATGATCGAT	54	ATTGTTGTGGACATTTGAGCG	73	AACTGCAGCATTTAGGGAA
13	CGAGAGTGCAGGAAGAAAGAA	32	TGAAACTAAGTCTTTTACCA	55	ACATTCAGCGTGTGGAAGC	74	AGCTCTGCAAACTGCGACA
14	GAAGAAAGAAAGAACTCGAG	33	AACTGGAAAGTGAAGTAAAG	56	TGATGGAAAGCTTCATAATGA	75	GAATCCGCAAGCTCTGCA
15	GAATCCGAGAGCTATTCA	34	ACACAAAAGAACTGGAAAC	57	TTCAATAATGAACGTTCAAGA	76	CTCGAGCTGGAATCCCGCA
16	AAGCTATTTCAGACAAATGCTG	35	CCAAATTAACACACAAAGA	58	ACGTTCAAGAGCTGGCCACA	77	CTTCGCTACTCTGCGAGCTG
17	GACATGCTGGATACACCCAG	36	ACTGACAAACCCAAATTACC	59	GCTGGCCACAACTCCGTGTAG	78	GTTCTTCTTCTTCGTCAT
18	GATACACCGAGGACCATACCT	37	TAGTACAAACCACTGACAAAC	60	ATCGGTGTAGAGAACTTCC	79	GCTTCTCGGAGTTCTTCTTT
19	GACCATACCTTTGGATGTG	38	CAACCAAAAGTAGTACAACA	61	AGAACTCTTCTGTGAAGATA	80	TGTCTGAATAGCTTCTCGGA
20	GTTGATGTGATATGTCGCG	39	CGTCTGGGAACAACCAAAAG	62	TGTGAAGATATCTTGTAA	81	TATCCAGCATTTGTGAATA
21	ATATGTCGCGACCAAGAACA	40	ACAGCGAGTACGCTGTGGAA	63	CTCTTGTAAACAACCAAGCA	82	GCTCCAGGTATATCCAGCAT
22	ACCAAGAACATGTTTACCG	41	CAAAACAACAACACGCGAGTA	64	ACAACAGCATCTTTGGATG	83	CAACAGGTACGGTCCAGGTG
23	TGTGTTACCGATGATCCAA	42	AAAAGGAAACAAAACAACA	65	TCCTTGGGATGCTCATCAAT	84	AGATGACATCCAAACAGGTAC
43	ATGATCCCAAGTGGTGGCACT	44	AGTGCCACCACTTGGGATCAT	85	GTCATGCAATGGGAAGATCGG	86	CCGATCTTCCCAATTGCATGAC
45	ATGATCCCAATGGTGGCACT	46	AGTGCCACCACTTGGGATCAT	87	GTCATGCAATGGGAAGATCGG	88	CCGATCTTCCCAATTGCATGAC

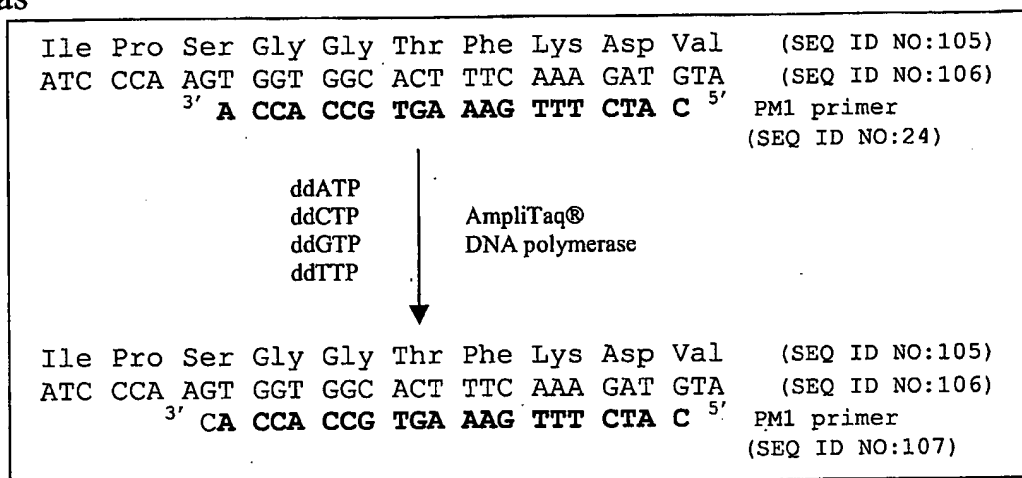
All oligonucleotides noted as being in the forward direction are located on the 5' side of either PM1 or PM2 mutation, in reference to SEQ ID:1 and SEQ ID:2. All oligonucleotides noted as being in the reverse direction are located on the 3' side of either the PM1 or PM2 mutation, in reference to SEQ ID:3 and SEQ ID:4.

FIG. 1E

“PM1” Test

AHAS1

‘Topas’



‘PM1’

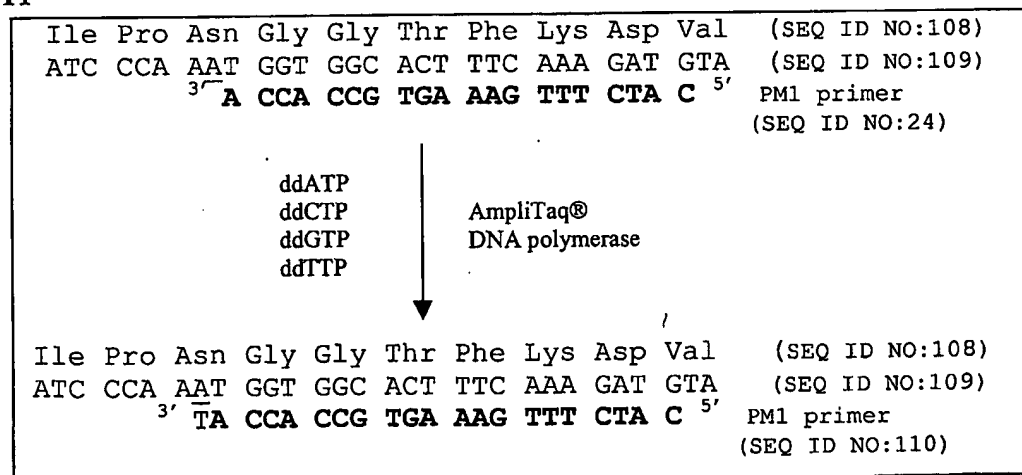
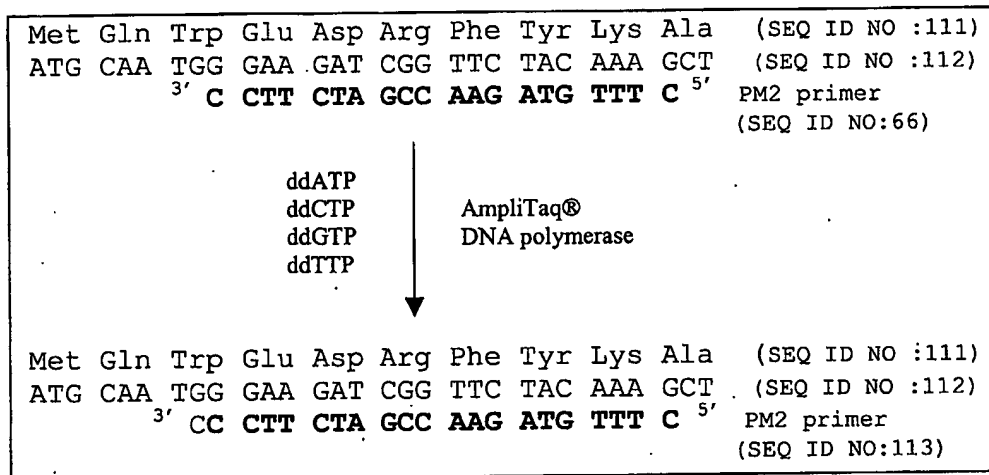


FIG. 2

“PM2” Test

AHAS3

‘Topas’



‘PM2’

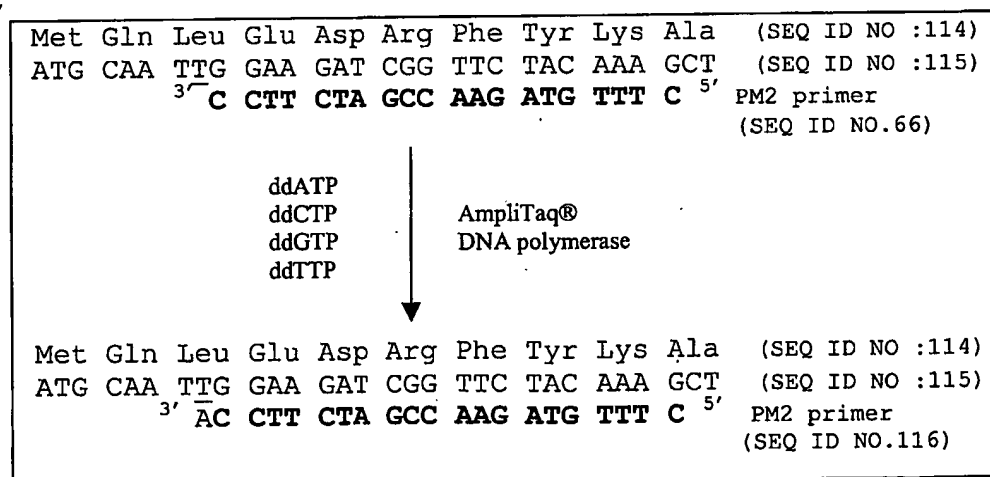


FIG. 3

Doubled Haploid Canola Lines		
Plant number	class	GH Rating
1	"PM1/PM2"	0
2	"PM1/PM2"	0
3	"PM1/PM2"	0
4	"PM1/PM2"	0
6	"PM1/PM2"	5
7	"PM1/PM2"	5
37	"PM2"	20
38	"PM2"	20
39	"PM2"	20
40	"PM2"	25
41	"PM2"	25
42	"PM2"	25
68	"PM1"	40
69	"PM1"	40
70	"PM1"	40
71	"PM1"	40
72	"PM1"	40
73	"PM1"	45
103	WT	80
104	WT	80
105	WT	80
106	WT	80
107	WT	85
108	WT	85
-	"PM1"	-
-	"PM2"	-
-	W T	-

FIG. 4

Summary of results from "PM1" and "PM2" mutation tests with three replicates using the ABI PRISM Technology

DNA class	Plant number ²	"PM1" mutation test		"PM2" mutation test	
		Observed ³	Expected ⁴	Observed ³	Expected ⁴
"PM1/PM2" class	1	(C)-T	C-T	(C)-A	C-A
	2	(C)-T	C-T	(C)-A	C-A
	3	(C)-T	C-T	(C)-A	C-A
	4	(C)-T	C-T	(C)-A	C-A
	6	(C)-T	C-T	(C)-A	C-A
	7	(C)-T	C-T	(C)-A	C-A
	37	(C)-T	C	(C)-A	C-A
	38	(C)-T	C	(C)-A	C-A
"PM2" class	39	(C)-T-(A)	C	(C)-A	C-A
	40	C	C	(C)-A	C-A
	41	(C)-T	C	(C)-A	C-A
	42	(C)-T	C	(C)-A	C-A
	68	T	C-T	C	C
	69	T	C-T	C	C
	70	T	C-T	C	C
	71	T	C-T	C	C
"PM1" class	72	T	C-T	C	C
	73	T	C-T	C	C
	103	C	C	C	C
	104	C	C	C	C
	105	C	C	C	C
	106	C	C	C	C
	107	C	C	C	C
	108	C	C	C	C
"PM1" control ¹	-	(C)-T	C-T	C	C
"PM2" control ¹	-	C	C	C-A	C-A
"Topas" (WT) control ¹	-	C	C	C	C

¹PM1 control and PM2 control are DNA isolated from PM1 and PM2 plants used to develop the tests.

²This number refers to the plant number for DH line identified in Figure 4

³In Bold and *italics* are the peaks related to the mutations and in brackets are the peaks that are not always present in all the three replicates.

⁴Expected results assuming that the AHAS amplification on reaction using AHAS1/AHAS3 amplification primers (SED ID NO:90 and 91) amplified similar amounts of both AHAS1 and AHAS3 sequences and that the PM1 extension primers will anneal also to the AHAS3 sequence and the PM2 extension primers will anneal also to the AHAS1 sequence.

FIG. 5

Summary of results from "PM1" and "PM2" mutation tests using the Pyrosequencing PSQ 96 Technology

		"PM1" mutation test		"PM2" mutation test			
DNA sample	Pyrosequencing results	SEQ ID NO	"PM1" Mutation	Pyrosequencing results	SEQ ID NO	"PM2" Mutation	
'PM1'	CAAATGGTGG	98	Yes	GGGAAGATC	99	No	
'PM2'	CAAGTGGTGG	97	No	TGGGAAGATC	100	Yes	
'Topas' (WT)	CAAGTGGTGG	97	No	GGGAAGATC	99	No	

FIG. 6

SEQUENCE LISTING

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<120> COMPOSITIONS AND METHODS FOR IDENTIFYING PLANTS HAVING
INCREASED TOLERANCE TO IMIDAZOLINONE HERBICIDES

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Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser Pro Leu Pro Ile Ser
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aga ttc tcc ctt ccc ttc tcc tta acc cca cag aaa gac tcc tcc cgt      147
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gtc gca cct cct tcc cct gaa aaa acc gac aag aac aag act ttc gtc      243
Val Ala Pro Pro Ser Pro Glu Lys Thr Asp Lys Asn Lys Thr Phe Val
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 Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Ser Thr Ile
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 Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala Met Leu
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 Asp Ser Val Pro Leu Val Ala Ile Thr Gly Gln Val Pro Arg Arg Met
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 Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val Glu Val Thr Arg
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 Thr Ser Ser Ser Pro Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser
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cct cta ccc att tcc aga ttc tcc ctt ccc ttc tcc tta acc cca cag 150
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 Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile Ser Ala Val Leu
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 Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala Met Leu
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 Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val Arg Phe Asp Asp
 350 355 360

cgt gtc acg gga aag ctc gag gct ttc gct agc agg gct aaa att gtg 1155
 Arg Val Thr Gly Lys Leu Glu Ala Phe Ala Ser Arg Ala Lys Ile Val
 365 370 375

cac ata gac att gat tct gct gag att ggg aag aat aag aca cct cac 1203
 His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr Pro His
 380 385 390

gtg tct gtg tgt ggt gat gta aag ctg gct ttg caa ggg atg aac aag 1251
 Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln Gly Met Asn Lys
 395 400 405

gtt ctt gag aac cgg gcg gag gag ctc aag ctt gat ttc ggt gtt tgg 1299
 Val Leu Glu Asn Arg Ala Glu Glu Leu Lys Leu Asp Phe Gly Val Trp
 410 415 420 425

agg agt gag ttg agc gag cag aaa cag aag ttc cct ttg agc ttc aaa 1347
 Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys Phe Pro Leu Ser Phe Lys
 430 435 440

acg ttt gga gaa gcc att cct ccg cag tac gcg att cag atc ctc gac 1395
 Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile Gln Ile Leu Asp
 445 450 455

gag cta acc gaa ggg aag gca att atc agt act ggt gtt gga cag cat 1443
 Glu Leu Thr Glu Gly Lys Ala Ile Ile Ser Thr Gly Val Gly Gln His
 460 465 470

cag atg tgg gcg gcg cag ttt tac aag tac agg aag ccg aga cag tgg 1491
 Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr Arg Lys Pro Arg Gln Trp
 475 480 485

ctg tcg tca tca ggc ctc gga gct atg ggt ttt gga ctt cct gct gcg 1539
 Leu Ser Ser Ser Gly Leu Gly Ala Met Gly Phe Gly Leu Pro Ala Ala
 490 495 500 505
 att gga gcg tct gtg gcg aac cct gat gcg att gtt gtg gat att gac 1587
 Ile Gly Ala Ser Val Ala Asn Pro Asp Ala Ile Val Val Asp Ile Asp
 510 515 520
 ggt gat gga agc ttc ata atg aac gtt caa gag ctg gcc aca atc cgt 1635
 Gly Asp Gly Ser Phe Ile Met Asn Val Gln Glu Leu Ala Thr Ile Arg
 525 530 535
 gta gag aat ctt cct gtg aag ata ctc ttg tta aac aac cag cat ctt 1683
 Val Glu Asn Leu Pro Val Lys Ile Leu Leu Leu Asn Asn Gln His Leu
 540 545 550
 ggg atg gtc atg caa tgg gaa gat cgg ttc tac aaa gct aac aga gct 1731
 Gly Met Val Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala
 555 560 565
 cac act tat ctc ggg gac ccg gca agg gag aac gag atc ttc cct aac 1779
 His Thr Tyr Leu Gly Asp Pro Ala Arg Glu Asn Glu Ile Phe Pro Asn
 570 575 580 585
 atg ctg cag ttt gca gga gct tgc ggg att cca gct gcg aga gtg acg 1827
 Met Leu Gln Phe Ala Gly Ala Cys Gly Ile Pro Ala Ala Arg Val Thr
 590 595 600
 aag aaa gaa gaa ctc cga gaa gct att cag aca atg ctg gat aca cca 1875
 Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln Thr Met Leu Asp Thr Pro
 605 610 615
 gga cca tac ctg ttg gat gtg ata tgt ccg cac caa gaa cat gtg tta 1923
 Gly Pro Tyr Leu Leu Asp Val Ile Cys Pro His Gln Glu His Val Leu
 620 625 630
 ccg atg atc cca agt ggt ggc act ttc aaa gat gta ata aca gaa ggg 1971
 Pro Met Ile Pro Ser Gly Gly Thr Phe Lys Asp Val Ile Thr Glu Gly
 635 640 645
 gat ggt cgc act aag tac tgagagatga agctggtgat cgatcatatg 2019
 Asp Gly Arg Thr Lys Tyr
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<223> a, c, g, t, other or unknown

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 Thr Ser Pro Ser Pro Ile Ser Leu Thr Ala Lys Pro Ser Ser Lys Ser
 5 10 15 20

cct cta ccc att tcc aga ttc tcc ctt ccc ttc tcc tta acc cca cag 150
 Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser Leu Thr Pro Gln
 25 30 35

aaa ccc tcc tcc cgt ctc cac cgt cca ctc gcc atc tcc gcc gtt ctc 198
 Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile Ser Ala Val Leu
 40 45 50

aac tca ccc gtc aat gtc gca cct gaa aaa acc gac aag atc aag act 246
 Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp Lys Ile Lys Thr
 55 60 65

ttc atc tcc cgc tac gct ccc gac gag ccc cgc aag ggt gct gat atc 294
 Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys Gly Ala Asp Ile
 70 75 80

ctc gtg gaa gcc ctc gag cgt caa ggc gtc gaa acc gtc ttc gct tat 342
 Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr Val Phe Ala Tyr
 85 90 95 100

ccc gga ggt gcc tcc atg gag atc cac caa gcc ttg act cgc tcc tcc 390
 Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Ser
 105 110 115

acc atc cgt aac gtc ctc ccc cgt cac gaa caa gga gga gtc ttc gcc 438
 Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly Gly Val Phe Ala
 120 125 130

gcc gag ggt tac gct cgt tcc tcc ggc aaa ccg gga atc tgc ata gcc 486
 Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala
 135 140 145

act tcg ggt ccc gga gct acc aac ctc gtc agc ggg tta gcc gac gcg 534
 Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala Asp Ala
 150 155 160

atg ctt gac agt gtt cct ctc gtc gcc atc aca gga cag gtc cct cgc 582
 Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly Gln Val Pro Arg
 165 170 175 180

cgg atg atc ggt act gac gcg ttc caa gag acg cca atc gtt gag gta 630
 Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val Glu Val
 185 190 195

acg agg tct att acg aaa cat aac tat ctg gtg atg gat gtt gat gac 678
 Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val Asp Asp
 200 205 210

ata cct agg atc gtt caa gaa gca ttc ttt cta gct act tcc ggt aga 726
 Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala Thr Ser Gly Arg
 215 220 225

ccc gga ccg gtt ttg gtt gat gtt cct aag gat att cag cag cag ctt 774
 Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile Gln Gln Gln Leu
 230 235 240

gcg att cct aac tgg gat caa cct atg cgc ttg cct ggc tac atg tct 822
 Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro Gly Tyr Met Ser
 245 250 255 260

agg ctg cct cag cca ccg gaa gtt tct cag tta ggc cag atc gtt agg 870
 Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly Gln Ile Val Arg
 265 270 275

ttg atc tcg gag tct aag agg cct gtt ttg tac gtt ggt ggt gga agc 918
 Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val Gly Gly Gly Ser
 280 285 290

ttg aac tcg agt gag gaa ctg ggg aga ttt gtc gag ctt act ggg atc 966
 Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu Leu Thr Gly Ile
 295 300 305

cct gtt gcg agt acg ttg atg ggg ctt ggc tct tat cct tgt aac gat 1014
 Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr Pro Cys Asn Asp
 310 315 320

gag ttg tcc ctg cag atg ctt ggc atg cac ggg act gtg tat gct aac 1062
 Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr Val Tyr Ala Asn
 325 330 335 340

tac gct gtg gag cat agt gat ttg ttg ctg gcg ttt ggt gtt agg ttt 1110
 Tyr Ala Val Glu His Ser Asp Leu Leu Ala Phe Gly Val Arg Phe
 345 350 355

gat gac cgt gtc acg gga aag ctc gag gcg ttt gcg agc agg gct aag 1158
 Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala Ser Arg Ala Lys
 360 365 370

att gtg cac ata gac att gat tct gct gag att ggg aag aat aag aca 1206
 Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr
 375 380 385

cct cac gtg tct gtg tgt ggt gat gta aag ctg gct ttg caa ggg atg 1254
 Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln Gly Met
 390 395 400

aac aag gtt ctt gag aac cgg gcg gag gag ctc aag ctt gat ttc ggt 1302
 Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Lys Leu Asp Phe Gly
 405 410 415 420

gtt tgg agg agt gag ttg agc gag cag aaa cag aag ttc ccg ttg agc 1350
 Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys Phe Pro Leu Ser
 425 430 435

ttc aaa acg ttt gga gaa gcc att cct ccg cag tac gcg att cag gtc 1398
 Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile Gln Val
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cta gac gag cta acc caa ggg aag gca att atc agt act ggt gtt gga 1446
 Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Ser Thr Gly Val Gly
 455 460 465

cag cat cag atg tgg gcg gcg cag ttt tac aag tac agg aag ccg agg 1494
 Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr Arg Lys Pro Arg
 470 475 480

cag tgg ctg tcg tcc tca gga ctc gga gct atg ggt ttc gga ctt cct 1542
 Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly Phe Gly Leu Pro
 485 490 495 500

gct gcg att gga gcg tct gtg gcg aac cct gat gcg att gtt gtg gac 1590
 Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala Ile Val Val Asp
 505 510 515

att gac ggt gat gga agc ttc ata atg aac gtt caa gag ctg gcc aca 1638
 Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln Glu Leu Ala Thr
 520 525 530

atc cgt gta gag aat ctt cct gtg aag ata ctc ttg tta aac aac cag 1686
 Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu Leu Asn Asn Gln
 535 540 545

cat ctt ggg atg gtc atg caa tgg gaa gat cgg ttc tac aaa gct aac 1734
 His Leu Gly Met Val Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn
 550 555 560

aga gct cac act tat ctc ggg gac ccg gca agg gag aac gag atc ttc 1782
 Arg Ala His Thr Tyr Leu Gly Asp Pro Ala Arg Glu Asn Glu Ile Phe
 565 570 575 580

cct aac atg ctg cag ttt gca gga gct tgc ggg att cca gct gcg aga 1830
 Pro Asn Met Leu Gln Phe Ala Gly Ala Cys Gly Ile Pro Ala Ala Arg
 585 590 595

gtg acg aag aaa gaa gaa ctc cga gaa gct att cag aca atg ctg gat 1878
 Val Thr Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln Thr Met Leu Asp
 600 605 610

aca cct gga ccg tac ctg ttg gat gtc atc tgt ccg cac caa gaa cat 1926
 Thr Pro Gly Pro Tyr Leu Leu Asp Val Ile Cys Pro His Gln Glu His
 615 620 625

gtg tta ccg atg atc cca agt ggt ggc act ttc gaa gat gta ata acc 1974
 Val Leu Pro Met Ile Pro Ser Gly Gly Thr Phe Glu Asp Val Ile Thr
 630 635 640

gaa ggg gat ggt cgc act aag tac tgagagatga agctggtgat ccatcatatg 2028
 Glu Gly Asp Gly Arg Thr Lys Tyr
 645 650

gtaaaagact tagtttcagt ttacagtttc tttgtgtgg taatttggt ttgtcagttg 2088
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<210> 6

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<223> Description of Artificial Sequence: Synthetic
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<210> 7
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<210> 8
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<223> Description of Artificial Sequence: Synthetic
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<400> 11
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<210> 12
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<210> 14
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<210> 15
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<210> 17
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<210> 24
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<210> 25
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<400> 25
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<210> 29
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<400> 30
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<210> 31
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<400> 32
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<210> 33
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<210> 34
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oligonucleotide

<400> 34
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<400> 35
ccaaattacc acacaaaaga

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oligonucleotide

<400> 36
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<400> 37
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<210> 38
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<400> 38
caaccaaaag tagtacaaca

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<210> 39
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<210> 40
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<400> 41
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<400> 42
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<400> 43
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21

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<400> 44
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<210> 45
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<400> 45
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21

<210> 46
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oligonucleotide

<400> 46
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21

<210> 47
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<210> 48
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oligonucleotide

<400> 48
ggagctatgg gtttcggact

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<210> 49
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<212> DNA
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oligonucleotide

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gtttcggact tcctgctgcg

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<210> 50
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<400> 50
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<210> 51
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<210> 52
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<210> 53
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<400> 53
ccctgatgcg attgttgtgg 20

<210> 54
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<400> 54
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<210> 55
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<210> 56
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<210> 57
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<210> 58
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<210> 59
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<223> Description of Artificial Sequence: Synthetic
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<210> 60
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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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<210> 61
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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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agaattcttc tgtgaagata

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<210> 62
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<223> Description of Artificial Sequence: Synthetic
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<210> 63
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<400> 63
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<210> 64
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<400> 64
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<210> 65
<211> 20
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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 65
tcttgggatg gtcacgaat

20

<210> 66
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 66
ctttgtagaa ccgatcttcc

20

<210> 67
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 67
gctctgtag cttttagaa

20

<210> 68
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 68
ataagtgtga gctctgtag

20

<210> 69
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 69
ggtccccgag ataagtgtga

20

<210> 70
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 70
tcccttgccg ggtccccgag

20

<210> 71
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 71
gatctcggtc tcccttgccg

20

<210> 72
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 72
tgtagggaa gatctcggtc

20

<210> 73
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 73
aactgcagca tgtagggaa

20

<210> 74
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 74
agtcctgca aactgcagca

20

<210> 75
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 75
gaatcccgcga agctcctgca

20

<210> 76
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 76
ctcgagctg gaatcccgcga

20

<210> 77
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 77
cttcgtcact ctcgagctg

20

<210> 78
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 78
gttcttcttt cttcgtcact

20

<210> 79
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 79
gcttctcgga gttcttcttt

20

<210> 80
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 80
tgtctgaata gcttctcgga

20

<210> 81
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 81
tatccagcat tgtctgaata

20

<210> 82
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 82
ggtccaggtg tatccagcat

20

<210> 83
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 83
caacaggtac ggtccaggtg

20

<210> 84
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 84
agatgacatc caacaggtac

20

<210> 85
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 85
gtcatgcaat gggaagatcg g

21

<210> 86
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 86
ccgatcttcc cattgcatga c

<210> 87
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 87
gtcatgcaat tggaaagatcg g

21

<210> 88
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 88
ccgatcttcc aattgcatga c

21

<210> 89
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
primer

<400> 89
tacatctttg aaagtgccca

19

<210> 90
<211> 21
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
primer

<400> 90
ggcgtttggt gttaggtttg a

21

<210> 91
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 91
cgtctgggaa caaccaaaag t

21

<210> 92
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 92
ggaaagctcg aggctttcgc t

21

<210> 93
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 93
atcaccagct tcattcttca gt

22

<210> 94
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 94
ggaaagctcg aggcgtttgc g

21

<210> 95
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 95
gtggtaccga tgatcc

16

<210> 96
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 96
gggatggtca tgcaat

16

<210> 97
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 97
caagtgggtgg

10

<210> 98
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 98
caaatggtgg

10

<210> 99
 <211> 9
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 99
 gggaagatc

9

<210> 100
 <211> 9
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic primer

<400> 100
 tggaagatc

9

<210> 101
 <211> 655
 <212> PRT
 <213> Brassica napus

<220>
 <221> MOD_RES
 <222> (268)..(268)
 <223> Variable amino acid

<400> 101
 Met Ala Ala Ala Thr Ser Ser Ser Pro Ile Ser Leu Thr Ala Lys Pro
 1 5 10 15
 Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
 20 25 30
 Leu Thr Pro Gln Lys Asp Ser Ser Arg Leu His Arg Pro Leu Ala Ile
 35 40 45
 Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Pro Ser Pro Glu
 50 55 60
 Lys Thr Asp Lys Asn Lys Thr Phe Val Ser Arg Tyr Ala Pro Asp Glu
 65 70 75 80
 Pro Arg Lys Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly
 85 90 95

Val Glu Thr Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His
 100 105 110
 Gln Ala Leu Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His
 115 120 125
 Glu Gln Gly Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly
 130 135 140
 Lys Pro Gly Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu
 145 150 155 160
 Val Ser Gly Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala
 165 170 175
 Ile Thr Gly Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln
 180 185 190
 Glu Thr Pro Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr
 195 200 205
 Leu Val Met Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe
 210 215 220
 Phe Leu Ala Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro
 225 230 235 240
 Lys Asp Ile Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met
 245 250 255
 Arg Leu Pro Gly Tyr Met Ser Arg Leu Pro Gln Xaa Pro Glu Val Ser
 260 265 270
 Gln Leu Gly Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val
 275 280 285
 Leu Tyr Val Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg
 290 295 300
 Phe Val Glu Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu
 305 310 315 320
 Gly Ser Tyr Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met
 325 330 335
 His Gly Thr Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu
 340 345 350
 Leu Ala Phe Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu
 355 360 365
 Ala Phe Ala Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala
 370 375 380

Glu Ile Gly Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val
 385 390 395 400
 Lys Leu Ala Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu
 405 410 415
 Glu Leu Lys Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln
 420 425 430
 Lys Gln Lys Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro
 435 440 445
 Pro Gln Tyr Ala Ile Gln Ile Leu Asp Glu Leu Thr Glu Gly Lys Ala
 450 455 460
 Ile Ile Ser Thr Gly Val Gly Gln Arg Gln Met Trp Ala Ala Gln Phe
 465 470 475 480
 Tyr Lys Tyr Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly
 485 490 495
 Ala Met Gly Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn
 500 505 510
 Pro Asp Ala Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met
 515 520 525
 Asn Val Gln Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys
 530 535 540
 Ile Leu Leu Leu Asn Asn Gln His Leu Gly Met Val Met Gln Trp Glu
 545 550 555 560
 Asp Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro
 565 570 575
 Ala Arg Glu Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala
 580 585 590
 Cys Gly Ile Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu
 595 600 605
 Ala Ile Gln Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Val
 610 615 620
 Ile Cys Pro His Gln Glu His Val Leu Pro Met Ile Pro Asn Gly Gly
 625 630 635 640
 Thr Phe Lys Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
 645 650 655

<210> 102
 <211> 652
 <212> PRT
 <213> Brassica napus

<220>
 <221> MOD_RES
 <222> (464)..(464)
 <223> Variable amino acid

<400> 102
 Met Ala Ala Ala Thr Ser Ser Ser Pro Ile Ser Leu Thr Ala Lys Pro
 1 5 10 15
 Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
 20 25 30
 Leu Thr Pro Gln Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile
 35 40 45
 Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp
 50 55 60
 Lys Ile Lys Thr Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys
 65 70 75 80
 Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr
 85 90 95
 Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu
 100 105 110
 Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly
 115 120 125
 Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly
 130 135 140
 Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly
 145 150 155 160
 Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly
 165 170 175
 Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro
 180 185 190
 Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met
 195 200 205
 Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala
 210 215 220

Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile
 225 230 235 240
 Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro
 245 250 255
 Gly Tyr Met Ser Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly
 260 265 270
 Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val
 275 280 285
 Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu
 290 295 300
 Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr
 305 310 315 320
 Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr
 325 330 335
 Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu Leu Ala Phe
 340 345 350
 Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala
 355 360 365
 Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly
 370 375 380
 Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala
 385 390 395 400
 Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Leu Lys
 405 410 415
 Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys
 420 425 430
 Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr
 435 440 445
 Ala Ile Gln Val Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Xaa
 450 455 460
 Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr
 465 470 475 480
 Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly
 485 490 495
 Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala
 500 505 510

Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln
 515 520 525
 Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu
 530 535 540
 Leu Asn Asn Gln His Leu Gly Met Val Met Gln Leu Glu Asp Arg Phe
 545 550 555 560
 Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro Ala Arg Glu
 565 570 575
 Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala Cys Gly Ile
 580 585 590
 Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu Ala Ile Gln
 595 600 605
 Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Ala Ile Cys Pro
 610 615 620
 His Gln Glu His Val Leu Pro Met Ile Pro Ser Gly Gly Thr Phe Lys
 625 630 635 640
 Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
 645 650

<210> 103
 <211> 655
 <212> PRT
 <213> Brassica napus

<400> 103
 Met Ala Ala Ala Thr Ser Ser Ser Pro Ile Ser Leu Thr Ala Lys Pro
 1 5 10 15
 Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
 20 25 30
 Leu Thr Pro Gln Lys Asp Ser Ser Arg Leu His Arg Pro Leu Ala Ile
 35 40 45
 Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Pro Ser Pro Glu
 50 55 60
 Lys Thr Asp Lys Asn Lys Thr Phe Val Ser Arg Tyr Ala Pro Asp Glu
 65 70 75 80
 Pro Arg Lys Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly
 85 90 95
 Val Glu Thr Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His
 100 105 110

Gln Ala Leu Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His
 115 120 125
 Glu Gln Gly Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly
 130 135 140
 Lys Pro Gly Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu
 145 150 155 160
 Val Ser Gly Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala
 165 170 175
 Ile Thr Gly Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln
 180 185 190
 Glu Thr Pro Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr
 195 200 205
 Leu Val Met Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe
 210 215 220
 Phe Leu Ala Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro
 225 230 235 240
 Lys Asp Ile Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met
 245 250 255
 Arg Leu Pro Gly Tyr Met Ser Arg Leu Pro Gln Pro Pro Glu Val Ser
 260 265 270
 Gln Leu Gly Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val
 275 280 285
 Leu Tyr Val Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg
 290 295 300
 Phe Val Glu Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu
 305 310 315 320
 Gly Ser Tyr Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met
 325 330 335
 His Gly Thr Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu
 340 345 350
 Leu Ala Phe Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu
 355 360 365
 Ala Phe Ala Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala
 370 375 380
 Glu Ile Gly Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val
 385 390 395 400

Lys Leu Ala Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu
 405 410 415
 Glu Leu Lys Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln
 420 425 430
 Lys Gln Lys Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro
 435 440 445
 Pro Gln Tyr Ala Ile Gln Ile Leu Asp Glu Leu Thr Glu Gly Lys Ala
 450 455 460
 Ile Ile Ser Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Phe
 465 470 475 480
 Tyr Lys Tyr Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly
 485 490 495
 Ala Met Gly Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn
 500 505 510
 Pro Asp Ala Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met
 515 520 525
 Asn Val Gln Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys
 530 535 540
 Ile Leu Leu Leu Asn Asn Gln His Leu Gly Met Val Met Gln Trp Glu
 545 550 555 560
 Asp Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asp Pro
 565 570 575
 Ala Arg Glu Asn Glu Ile Phe Pro Asn Met Leu Gln Phe Ala Gly Ala
 580 585 590
 Cys Gly Ile Pro Ala Ala Arg Val Thr Lys Lys Glu Glu Leu Arg Glu
 595 600 605
 Ala Ile Gln Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu Leu Asp Val
 610 615 620
 Ile Cys Pro His Gln Glu His Val Leu Pro Met Ile Pro Ser Gly Gly
 625 630 635 640
 Thr Phe Lys Asp Val Ile Thr Glu Gly Asp Gly Arg Thr Lys Tyr
 645 650 655

<210> 104

<211> 652

<212> PRT

<213> Brassica napus

<400> 104

Met Ala Ala Ala Thr Ser Pro Ser Pro Ile Ser Leu Thr Ala Lys Pro
 1 5 10 15
 Ser Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu Pro Phe Ser
 20 25 30
 Leu Thr Pro Gln Lys Pro Ser Ser Arg Leu His Arg Pro Leu Ala Ile
 35 40 45
 Ser Ala Val Leu Asn Ser Pro Val Asn Val Ala Pro Glu Lys Thr Asp
 50 55 60
 Lys Ile Lys Thr Phe Ile Ser Arg Tyr Ala Pro Asp Glu Pro Arg Lys
 65 70 75 80
 Gly Ala Asp Ile Leu Val Glu Ala Leu Glu Arg Gln Gly Val Glu Thr
 85 90 95
 Val Phe Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln Ala Leu
 100 105 110
 Thr Arg Ser Ser Thr Ile Arg Asn Val Leu Pro Arg His Glu Gln Gly
 115 120 125
 Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg Ser Ser Gly Lys Pro Gly
 130 135 140
 Ile Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val Ser Gly
 145 150 155 160
 Leu Ala Asp Ala Met Leu Asp Ser Val Pro Leu Val Ala Ile Thr Gly
 165 170 175
 Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro
 180 185 190
 Ile Val Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Met
 195 200 205
 Asp Val Asp Asp Ile Pro Arg Ile Val Gln Glu Ala Phe Phe Leu Ala
 210 215 220
 Thr Ser Gly Arg Pro Gly Pro Val Leu Val Asp Val Pro Lys Asp Ile
 225 230 235 240
 Gln Gln Gln Leu Ala Ile Pro Asn Trp Asp Gln Pro Met Arg Leu Pro
 245 250 255
 Gly Tyr Met Ser Arg Leu Pro Gln Pro Pro Glu Val Ser Gln Leu Gly
 260 265 270

Gln Ile Val Arg Leu Ile Ser Glu Ser Lys Arg Pro Val Leu Tyr Val
 275 280 285
 Gly Gly Gly Ser Leu Asn Ser Ser Glu Glu Leu Gly Arg Phe Val Glu
 290 295 300
 Leu Thr Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu Gly Ser Tyr
 305 310 315 320
 Pro Cys Asn Asp Glu Leu Ser Leu Gln Met Leu Gly Met His Gly Thr
 325 330 335
 Val Tyr Ala Asn Tyr Ala Val Glu His Ser Asp Leu Leu Leu Ala Phe
 340 345 350
 Gly Val Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu Ala Phe Ala
 355 360 365
 Ser Arg Ala Lys Ile Val His Ile Asp Ile Asp Ser Ala Glu Ile Gly
 370 375 380
 Lys Asn Lys Thr Pro His Val Ser Val Cys Gly Asp Val Lys Leu Ala
 385 390 395 400
 Leu Gln Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu Glu Leu Lys
 405 410 415
 Leu Asp Phe Gly Val Trp Arg Ser Glu Leu Ser Glu Gln Lys Gln Lys
 420 425 430
 Phe Pro Leu Ser Phe Lys Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr
 435 440 445
 Ala Ile Gln Val Leu Asp Glu Leu Thr Gln Gly Lys Ala Ile Ile Ser
 450 455 460
 Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Phe Tyr Lys Tyr
 465 470 475 480
 Arg Lys Pro Arg Gln Trp Leu Ser Ser Ser Gly Leu Gly Ala Met Gly
 485 490 495
 Phe Gly Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn Pro Asp Ala
 500 505 510
 Ile Val Val Asp Ile Asp Gly Asp Gly Ser Phe Ile Met Asn Val Gln
 515 520 525
 Glu Leu Ala Thr Ile Arg Val Glu Asn Leu Pro Val Lys Ile Leu Leu
 530 535 540
 Leu Asn Asn Gln His Leu Gly Met Val Met Gln Trp Glu Asp Arg Phe
 545 550 555 560

<210> 108
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 peptide

<400> 108
 Ile Pro Asn Gly Gly Thr Phe Lys Asp Val
 1 5 10

<210> 109
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 primer

<400> 109
 atcccaaatg gtggcacttt caaagatgta

30

<210> 110
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic
 primer

<400> 110
 catctttgaa agtgccacca t

21

<210> 111
 <211> 10
 <212> PRT
 <213> Brassica napus

<400> 111
 Met Gln Trp Glu Asp Arg Phe Tyr Lys Ala
 1 5 10

<210> 112
 <211> 30
 <212> DNA
 <213> Brassica napus

<400> 112
atgcaatggg aagatcgggt ctacaaagct

30

<210> 113
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 113
ctttgtagaa ccgatcttcc c

21

<210> 114
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 114
Met Gln Leu Glu Asp Arg Phe Tyr Lys Ala
1 5 10

<210> 115
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

<400> 115
atgcaattgg aagatcgggt ctacaaagct

30

<210> 116
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
primer

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<400> 116
ctttgtagaa ccgatcttcc a.

21